CONTAMINATION LEVELS IN AND CELLULAR RESPONSES OF INTERTIDAL INVERTEBRATES AS BIOMARKERS OF TOXIC STRESS CAUSED BY HEAVY METAL CONTAMINATION IN FALSE BAY

BY

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work that I have not previously, in its entirety or in part, submitted at any university for a degree.

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ABSTRACT

Heavy metals are persistent environmental contaminants whose sources of inputs into the environment are both natural and anthropogenic. The levels of heavy metals (cadmium, copper, nickel, lead and zinc) in the False Bay intertidal zone were measured in the water, sediments and invertebrate species between August 2000 and August 2001. The results of the water and sediment analyses revealed that most pollution was associated with the northern shore of the bay between Strand and Muizenberg, where the most populated and industrial catchments occur. Significant spatial variations occurred, indicating the presence of localised contamination, while seasonal variations may be related to changes in precipitation and runoff at different times of the year. The concentrations of cadmium, nickel and lead were occasionally higher than the levels recommended by the South African Water Quality Standards. The possible sources of pollution at the different sites are also discussed. The concentrations of the five metals in the different invertebrate species (*Oxystele tigrina, O. sinensis, Choromytilus meridionalis, Patella oculus, Patiriella exigua* and *Tetraclita serrata)* also revealed significant seasonal and spatial variations, with both the soft tissues and shells accumulating heavy metals. The barnacle *T. serrata* from Rooiels had the highest cadmium concentration (70.67 μg/g dry weight), which may be related to historic pollution inputs from the military activities which took place at a weapons testing site at this site between 1987 and 1994, although no evidence was found to confirm this. The periwinkle *O. tigrina* from Strand had the highest copper concentration (70.25 μg/g) while the limpet *P. oculus* from the same site had the highest nickel concentration (35.75 μg/g). The shells of the mussel *C. meridionalis* from Muizenberg had the highest concentration of lead (25.75 μg/g). Since cadmium occurs as a constituent of phosphate fertilisers used widely in the False Bay catchments, the effects of cadmium exposure on the different species were investigated during 14-day laboratory exposures to 200 and 400 μg/L $CdCl_2$. The results revealed a general pattern of tissue metal increase in the exposed organisms, followed by slight reductions after decontamination in clean seawater. The viscera and kidneys of *C. meridionalis* accumulated most of the dissolved cadmium. The shells of the mussels also accumulated cadmium, indicating the possible use of shells as a detoxification matrix.

Abstract

The loss of cadmium from the organisms which were exposed to the higher concentration was not significant, indicating that cadmium was more tightly bound and sequestered in the tissues of these organisms. The sub-cellular effects of contaminants on the haemolymph lysosomes of field-collected and cadmium-exposed organisms were investigated using the Neutral Red Retention (NRR) assay, while the changes in lysosomal sizes and digestive gland epithelial area were measured using computer-assisted image analysis. The field organisms which were collected during winter and summer had shorter NRR times and enlarged lysosomes, indicating lysosomal membrane destabilisation related to increased stress during these two seasons. Significant spatial differences in the lysosomal sizes of *O. tigrina* and *P. exigua* were observed, with those from Strand having slightly larger lysosomes than those of the same species from the other sites, while *C. meridionalis* and *P. oculus* from Rooiels also had larger lysosomes than those from other sites. These lysosomal enlargements in the organisms from Strand and Rooiels may be an indication of increased contamination levels at these sites. Exposure to cadmium in the laboratory resulted in a dose-dependent reduction in NRR times and lysosomal size enlargement. When the cadmium-exposed organisms were moved to clean seawater, there was a slight increase in the NRR times after a week's decontamination, indicating some degree of recovery of the lysosomal membrane integrity. There was, however, no reduction in lysosomal sizes of the exposed organisms, indicating that this period was not sufficient for recovery of this biomarker. The digestive gland epithelial areas of Strand, Muizenberg and Rooiels species, as well as those of cadmium-exposed organisms, were significantly reduced. It was concluded that these lysosomal stress response was a rapid response that could be measured quantitatively and used as a general biomarker of toxic stress caused by environmental contamination and heavy metal exposure.

OPSOMMING

Swaarmetale is persisterende omgewingskontaminante waarvan die insetbronne beide natuurlik of van menslike oorsprong kan wees. Die kontaminasievlakke van swaarmetale (kadmium, koper, nikkel, lood en sink) in die Valsbaai tussengetysone is in die water, sedimente en invertebraatspesies bepaal vanaf Augustus 2000 tot Augustus 2001. Voorlopige resultate van die water- en sedimentontledings het getoon dat die meeste besoedeling by die noordelikke oewer van die baai voorgekom het tussen Strand and Muizenberg, waar die mees digbewoonde en ge-industrialiseerde opvangsgebiede is. Betekenisvolle ruimtelike en seisoenale variasie het in die konsentrasies van swaarmetale voorgekom, met die ruimtelike variasie wat moontlik gelokaliseerde kontaminasie aandui terwyl die seisoenale variasies weer verband mag hou met veranderings in die neerslag en afloop gedurende verskillende tye van die jaar. Die konsentrasie van kadmium, nikkel en lood was somtyds hoër as die vlakke wat deur die Suid-Afrikaanse Waterkwaliteitsstandaarde voorgestel word. Die moontlike bronne van besoedeling in die verskillende areas is ook in bespreking genoem. Die konsentrasies van die vyf swaametale in die verskillende invertebraatspesies (*Oxystele tigrina, O. sinensis, Choromytilus meridionalis, Patella oculus, Patiriella exigua* and *Tetraclita serrata*) het ook seisoenale en ruimtelike variasies vertoon, die swaarmetale het in die sagte weefsel en skulpe van die invertebrate geakkumuleer. Die hoogste gemiddelde konsentrasie van kadmium (70.67 µg/g droë massa) is in die heel-liggaam monsters van die eendemossel *T. serrata* gemeet wat by Rooiels versamel is. Die vlakke mag verband hou met die oprigting en aktiwiteite van die wapentoetsingsaanleg in die opvanggebied van die Rooiels lokaliteit tussen 1987 en 1994, maar geen bewyse daarvan is gevind nie. Die tolletjie, *O. tigrina* wat in die lokaliteit by Strand versamel is het die hoogste gemiddelde konsentrasie koper gehad 70.25 µ/g droë massa), terwyl die klipmossel *P. oculus* by dieselfde versamelpunt die hoogste konsentrasie nikkel (35.75 µg/g droë massa) gehad het. Eksperimentele studies is ook uitgevoer op vier invertebraat spesies wat vir 14 dae in akwaria blootgestel is aan see-water met 200 en 400 µg/L $CdCl_2$, en daarna gedekontamineer is in skoon seewater.

Opsomming

Weefselspesifieke cadmium akkumulasie is in die verskillende spesies bepaal vir beide blootgestelde sowel as kontrole-organismes. Die resultate het getoon dat 'n algemene patroon van toename in weefselmetaal voorgekom het in die organismes, gevolg deur effense afnames in die konsentrasies van kadmium na dekontaminasie. Die sagteweefsel en niere van die mossel *C. meridionalis* het die meeste kadmium geakkumuleer, wat moontlik die gevolg is van opname van die metaal saam met water in die spysverteringstelsel. Die skulp van die mossel wat aan die hoër konsentrasies van $CdCl_2$ blootgestel is, het ook die meeste kadmium ge-akkumuleer. Dit mag 'n aanduiding wees dat die skulp as 'n detoksifisereingsmatriks gebruik word. Die verlies aan kadmium vanuit die sagte weefsel van die organismes wat aan die hoër konsentrasie van $CdCl_2$ was nie betekenisvol nie, wat 'n aanduiding mag wees dat die kadmium in die weefsels sterker aan die metallothioniene gebind was. Die verlies aan kadmium vanuit die sagte weefsel van die organismes wat aan die hoër konsentrasie van $CdCl_2$ was nie betekenisvol nie, wat 'n aanduiding mag wees dat die cadmium in die weefsels sterker aan die metallothioniene gebind was. Die effek van kontaminante op hemolimf lisosome is in die lewende selle van invertebrate vanaf die verskillende lokaliteite met die neutralrooi retensietyd (NRR) bestudeer, en die veranderinge in lisosoomgrootte en die epiteel van verteringskliere is bepaal. Die organismes wat gedurende winter en somer versamel is, het korter NRR-tye en groter lisosome gehad as dié wat gedurende ander tye van die jaar versamel is. Variasie het ook tussen lokaliteite voorgekom, met *O. tigrina* en *P. oculus* vanaf Strand wat effens groter lisosome gehad het as dié vanaf ander lokaliteite. *C. meridionalis* en *P. oculus* wat by Rooiels versamel is, het groter lisosome gehad as dié van ander lokaliteite. Die vergroting van lisosome in die organismes wat by Strand en Rooiels versamel is, mag 'n aanduiding wees van 'n toename in die kontaminasie in die gebiede. Blootstelling aan kadmium het 'n dosis-verwante vermindering in NRR tye tot gevolg gehad. Die dekontaminasie van die blootgestelde organismes in skoon see-water het tot 'n effense toename in NRR tye gelei. Dit dui op 'n mate van herstel van die lisosomale membraanstabiliteit.

Opsomming

Die feit dat 'n vermindering in die groottes nie plaasgevind het, mag 'n aanduiding wees dat een week nie voldoende was om herstel moontlik te maak nie. Die epiteeloppervlaktes van die spysverteringsklier van organismes wat by Strand, Muizenberg en Rooiels versamel is, sowel as die van die kadmium-blootgestelde organismes, is betekenisvol verlaag. Die gevolgtrekkings is dat die lisosomale stresrespons 'n snelle respons is wat kwantitatief gemeet kan word en wat as 'n algemene biomerker van toksiese stres, wat deur omgewingskontaminante en metaaalblootstelling veroorsaak word, gebruik kan word.

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DEDICATION

Climbing

Never look behind, girls, when you're on the way
Time's enough for that, girls, on some other day.
Though the way be long, girls, face it with a will.
Take your pack upon your back, and tramp, tramp along.
When you're near the top, girls, of the rugged way,
Never stop to look behind, but climb up the hill.
The prize is at the top, girls, waiting there until,
Patient, brave and steady girls have mounted up the hill.

(Adapted- Author unknown)

I dedicate this work to all the strong women out there, who continue to struggle in a man's world to bring some meaning and stability into their lives and to those of their children. To my best friend, mentor and role model, Prof. Nomusa Gwalla-Ogisi, one of the "women who run with the wolves"- many thanks for believing in me and for inspiring me to reach out for my dream.

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CHAPTER 1 – GENERAL INTRODUCTION

1.1. MARINE CONTAMINATION AND THE GLOBAL RESPONSE

Coastal areas are often characterised by increasing population growth, agricultural and industrial development, as well as reclamation of the coastal zone for residential and commercial uses (UNEP, 2003). As a result of all these pressures, coastal areas are facing serious problems such as habitat degradation, erosion and natural resource depletion (Prem, 2003). In addition, coastal communities are often plagued by socio-economic problems such as unemployment and poverty, thus not only are natural resources and biodiversity affected by unsustainable forms of resource utilisation, but also, the quality of life of coastal populations is affected (Prem, 2003).

Considering the multiple uses of the coastal zone, development in this area is often diverse and inevitable. As a result, thousands of chemicals used to meet the technological and economic needs of society continue to find their way into the coastal and marine environments (Vanasi et al. 1993), making these ecosystems the global sinks for many pollutants originating from land-based sources such as mine-tailing, military activities, untreated domestic effluents, pollutants from marine-based industries such as oil mining, and waste from shipping activities (Anon., 2003). The contamination of the coastal environment by these pollutants has become a major concern as they are often taken up by aquatic organisms (Beyer et al., 1996), which may result in their accumulation in the tissues of these organisms (Rainbow, 1995) and, consequently, the rendering of valuable marine resources unfit for human consumption (UNEP, 2003).

The initial global reaction to the environmental degradation resulting from pollution by contaminants was a reactive approach characterised by increasing clean-up efforts. The 1992 Earth Summit in Rio de Janeiro, Brazil, ushered in a new era of world cooperation against environmental degradation, and sustainable development became the main theme (Hinds, 2003). Chapter 17 of the Agenda 21 document from the Earth Summit calls for the protection of oceans, seas and coastal areas, while Chapter 20 calls for an environmentally-sound management of hazardous wastes (ME-4, 2003).

With about 35% of South Africa's population living in coastal areas (Anon., 2003), this has resulted in intense pressure from the competing demands for coastal resources, thus placing these ecosystems under threat. International trends and public pressure have largely contributed to the development and implementation of various legislation, policies and guidelines which aim to minimise or prevent the deterioration of the South African coastal and marine environments (DEAT, 1999). These include the Dumping at Sea Control Act, Act 73 of 1980, the International Convention of Pollution from Ships Act, Act 2 of 1986, the Sea Fisheries Act, Act 12 of 1988, which distinguish between, and lists the different categories of waste, prohibits the disposal at sea of some of them, and prescribes permit requirements for the disposal of others (DEAT, 1999). The Environmental Conservation Act, Act 73 of 1989 demands Environmental Impact Assessments for all proposed developments which may affect coastal and marine environments, while the South African Water Quality Guidelines sets out the water quality objectives for designated uses of coastal waters. The White Paper on Sustainable Coastal Development has been recently completed, and promises more effective management of the coastal and marine ecosystems (DEAT, 1999).

1.2. NEED FOR BIOLOGICAL MONITORING

The contamination of coastal marine environments by heavy metal contamination is of major concern, as it may lead to the destruction of valuable marine resources (Bu-Olayan & Subrahmanyam, 1998). Due to the increasing input of chemical contaminants into the aquatic environment, it has become necessary to develop sensitive and reliable methods to assess the impact of toxicants on aquatic organisms at an early stage of exposure (Mitchelmore et al., 1998).

The term "heavy metal" refers to the group of metals and metalloids with an atomic density greater than 6 g / cm, and is applied to elements such as cadmium, chromium, copper, silver, nickel, lead and zinc (Rainbow, 1997). The biological monitoring of the effects of heavy metals is of primary importance, not only for assessing the degree of pollution of coastal waters, but also to determine whether marine organisms do take up pollutants (Ruelas-Inzunza & Paez-Osuna, 1998). Biological monitoring involves the use of indicator species or communities, and involves the analysis of metals accumulated in animal tissues (Nicholson, 1999a). This process may show spatial and temporal variation in the bioavailability of contaminants in the marine environment (Rainbow, 1995), and may also help to identify the sources of such variation, as well help in the assessment of the environmental health of aquatic systems (Loeb & Spacie, 1994).

Marine organisms can accumulate heavy metals from the seawater, suspended particles, sediments and food chains (Blackmore, 2001). Due to their ability to accumulate heavy metals, potential biomonitors must show a net accumulation strategy for the metals in question (Kahle & Zauke, 2003), allowing for comparison to be made over time, and providing an integrated measure of toxicological significance of these heavy metals in the aquatic environment (Rainbow, 1995). The use of biomonitors also introduces physiological variables which are ignored when only analyses of trace metals in water and sediments are used (Phillips, 1976). Ideally, biomonitors should be sedentary, abundant, long-lived and large enough to provide enough tissue for analysis (Rainbow, 1995), and they must be tolerant of exposure to environmental variations.

According to Rainbow et al. (1990), the bioaccumulation of contaminants in the tissues of aquatic organisms is influenced by environmental factors such as salinity, pH, temperature, ambient metal concentration, food source, the position of the animal in the food chain, as well as its metabolism.

Most pollutants act in a chronic way and result in primarily sublethal effects (Kohler et al., 1999), thus making the monitoring of biological effects problematic due to the fact that sublethal effects are often difficult to determine. It is unlikely that any one species could be a universal indicator organism which is able to assess contamination in all available forms, as the different species have different affinities for metals and absorption may be from different sources (Loeb & Spacie, 1994). The choice of a suite of biomonitors, therefore, needs to consider potential sources of metals to biota (Rainbow, 1995), such as from surrounding seawater and suspended particles via suspension feeding, and from deposited food particles.

1.3. HEAVY METALS AND THEIR TOXIC EFFECTS

Whether essential or not, all trace metals are potentially toxic at a threshold bioavailability (Rainbow et al., 1990). Cadmium and lead are among the most potentially harmful heavy metals (Bu-Olayan & Subrahmanyam, 1998), and can be accumulated by marine invertebrates to high tissue concentrations (Yan et al., 1997). They attack sulfur bonds in enzymes of aquatic organisms thereby immobilizing them, and bind to cell membranes and affect transport processes. Cadmium is very toxic, with harmful effects on molluscs and crustaceans even at low concentrations (Nassiri et al., 1997), and enters the aquatic environment as a consequence of the natural breaking down of the geological matrix and anthropogenic processes such as mining and smelting of metals, automobile manufacturing, incineration of industrial wastes and application of sewage sludge to agricultural soils. Oral doses have been found to cause anaemia and growth repression in laboratory animals such as rats (Newberne, 1976). Cadmium is slowly excreted, and has a biological half-life of 30 years (Mance, 1987) and, since it has an atomic radius very similar to that of calcium (Sidoumou et al., 1997), cadmium may be taken up through the calcium channels. Lead is a highly toxic element with all known effects on biological systems being deleterious (Twiss & Thomas, 1998).

The dominant source of global contamination by lead for the past 50 years has been the use of lead organic compounds as motor vehicle fuel additives (McCally, 2002), although the metal is considered less of a problem now than it was in the past due to measures taken by various countries to phase it out as an anti-knock compound in gasoline engine fuels (GESAMP, 2001). However, lead is still commonly used in industrial paints due to its characteristic resistance against corrosion (OSHA, 2000), and in the manufacturing of batteries, plastics, cable sheathing and ammunition (McCally, 2002). Sources of lead are exhaust emissions from combustion engines and leaching of lead-based paints into stormwater drains (Hops, 1990).

Nickel, the third heavy metal studied, is a naturally ubiquitous metal in soil, water and air (Herkovits et al., 2000). Although it is an essential part of the biological system, the toxic action of Ni is due to its ability to replace essential metals in metallo-enzymes (Babukutty & Chacko, 1995) as well as its carcinogenic effects (OSHA, 2000). Sources of Ni in the environment are the combustion of coal and oil for power generation, waste incineration and sewage sludge, steel manufacturing and electroplating.

Copper and zinc, the other two heavy metals investigated in the present study, are metabolically essential elements (Botton et al., 1998). However, excessive amounts of these elements may lead to toxicity. Zinc contamination has been observed in most industrial areas, with major sources being metallurgic, chemical and paper manufacturing, fisheries industry, agricultural fertilizers and domestic waste landfills (Barcellos & Lacerda, 1994).

1.4. BIOMARKERS AS INDICATORS OF POLLUTION

Critics of the biomonitoring approach have suggested that, although the method gives a good indication of environmental metal concentration, actual biological damage is not elucidated (Nicholson, 1999a). Biomarkers are measurements of chemical, physiological and morphological alterations at the below-individual level resulting from exposure of organisms to xenobiotics (Van Gestel & van Brummelen, 1996). They are sensitive indicators that reveal that toxicants have entered the organism and have been distributed within its tissues, and they can be measured in the cells, tissues or body fluids of biomonitors (Depledge et al., 1995). Often, biomarkers are measurements of cellular or subcellular changes that reflect deleterious exposures to organisms (Nacci & Nelson, 1992). They are therefore important tools in the detection of various environmental stresses and to assess the effects of these to organisms. Biomarkers are employed to ascertain the health of the organism, and have been developed for use in conjunction with chemical monitoring (Nicholson, 1999a).

According to Cossu et al. (2000), the use of biomarkers allows for the early detection of biological changes which may result in physiological disturbances in the long-term, and is a promising approach in the assessment of ecosystem health. The attempt to relate biomarker responses of organisms to increasing pollutant exposure, both in the field and laboratory, offers potential for improving the ecological relevance of ecotoxicological test procedures (Depledge et al., 1995). The ecologically relevant effects must be exerted beyond the level of the individual, for example at the population, community or ecosystem levels (Anon., 2003). Ecologically relevant endpoints should reflect the important ecosystem attributes that are related to helping sustain ecosystem structure and function, and may include any level of organization.

There are two kinds of biomarkers, those that measure only exposure to a contaminant, and those that measure both exposure and toxic effects (Walker et al., 1996). According to Lynch & Wiseman (1996), general biomarkers respond to most types of environmental stressors while specific biomarkers respond to particular stressors. Lawrence & Herrera (2000) defined stress as the result of any environmental change that causes a decrease in the capacity for productivity in an organism. Stress, by requiring organisms to divert energy to somatic maintenance at the expense of growth and reproduction, may lead to significant reduction in fitness, and to subsequent deterioration of population viability and community composition (Polak et al., 2003). Since they have the advantage of being a measure of stress incurred in the organism (Walker et al., 1996), biomarkers give more information that is biologically relevant than chemical residue analyses do (Rees, 1993), hence these two methods should be used in an integrated way. Recently, biomarkers have been used increasingly as warning signals of pollution-induced stress and environmental deterioration (Naes et al., 1995). They are able to show that contaminants are present in the environment, and that they have reached the affected tissues in sufficient amounts to cause the observed responses (Walker et al., 1996). In all aquatic organisms, biomarker responses may be complicated by various environmental and life-cycle variables such as age, sex, size, seasonality and temperature (Mitchelmore et al., 1998).

Previous authors identified molecular biomarkers of exposure, such as the induction of cytochrome P4501A and metallothioneins (MT) (Livingstone, 1993; Leung & Furness, 1999); lysosomal membrane damage in mussel digestive cells (Lowe & Pipe, 1994); DNA strand breaks in sea stars (Everaarts et al., 1998) and mussels (Mitchelmore et al., 1998); heat shock proteins (HSP70) in centipedes (Pyza et al., 1997); DNA adducts in mussels (Mitchelmore et al., 1998), and alterations of acetycholinesterase (AChE) activity in fishes (Sancho et al., 2000). Cellular biomarkers may serve as early indicators of environmental disturbance and are precursors to further biological dysfunction at higher levels of functional complexity (Nicholson, 1999a). One of the earliest detectable cellular alterations of contaminant exposure is associated with lysosomes (Regoli, 1992).

These are multi-functional organelles found in all eukaryotic cells, and are involved in many cytological roles, including enzyme-mediated intracellular digestion and cellular homeostasis of metals through sequestration and storage of metal-binding metallothioneins (Nicholson, 1999a).

Lysosomal-response biomarkers that respond to chemical stress are used to understand the effects of metals on organisms (Reinecke & Reinecke, 1999). Lysosomal membranes are often the targets of injury by xenobiotics during their sequestration function, which is an important detoxification system for metals (Moore, 1985). There is considerable evidence that lysosomal integrity and function are compromised following exposure to contaminants (Lowe & Pipe, 1994). Many metals accumulate within lysosomes and influence their biochemical function, thus making these organelles the ideal starting point for the investigation of cellular responses as they are noted for their compartmentalization and accumulation of a variety of chemicals and metals (Moore, 1985). Some of the lysosomal responses to contaminant exposure that have been identified include hepatopancreatic epithelial reduction (Moore, 1985), and changes in contents and in fusion events (Moore, 1988). There is also increased frequency of secondary lysosomes, excessive build-up of lipofuscin, accumulation of unsaturated lipid in lysosomes (Lowe & Pipe, 1994), and changes in membrane integrity (Moore, 1990). According to Moore (1985), toxic substances react primarily with the lysosomal membrane and induce the changes that lead to destabilisation and increased permeability.

1.5. MOTIVATION FOR AND OBJECTIVES OF THE PRESENT STUDY

The consumption of seafood has been shown to be a significant pathway of heavy metal exposure among coastal populations that depend on the sea for the majority of their dietary proteins (Browne et al., 2001). Following incidents such as the outbreak of the Minamata disease in Japan from mercury-contaminated seafood (GESAMP, 2001a), the Stockholm Conference on Marine Policy responded by banning the production and use of some toxic substances. The Protocol on Heavy Metals was signed in 1998 under the framework of the Convention on Long-range Transboundary

Air Pollution (MSC-E, 2003), placing heavy metals such as cadmium, lead and mercury as metals of first priority. In many developed countries, the reduction of coastal sewage discharges and improved sewage treatment have helped to improve water quality (GESAMP, 2001a). In the developing world, however, the provision of basic sanitation, urban sewer systems and sewage treatment have not kept pace.

False Bay, in the Western Cape (Figure 1, Chapter 2, p 14), is situated 34^0 15′ S and 18^0 40′ E (Taljaard et al., 2000), and is deepest at the south entrance, gradually sloping to the northern shore (Day, 1970). The winter temperatures in the bay show little variation, with the temperature gradient decreasing to barely 0.5 °C difference between the surface and 30 cm water depth. Summer shows a well-established pattern of heating effects towards the east, while autumn shows a rapid reduction in temperatures and a slight influx of warmer water at the mouth of the bay (Atkins, 1970). The presence of the Cape Peninsula Mountain chain to the west and the Hottentots-Holland Mountains to the east has resulted in complex wind regimes and variations to prevail in False Bay (Grindley & Taylor, 1970). When the south-easterly winds blow in spring, warmer surface waters drift into False Bay from the Agulhas Bank, moving northwards in a clockwise direction, and then towards the western shores (Day, 1970). As summer advances, the south-easterly winds become stronger, but as it abates during late summer, a marked thermocline occurs (Day, 1970).

The False Bay coastline, with its mixed shores consisting of sandy and rocky shoreline, and a multitude of topographical characteristics (Brown et al., 1991), presents a unique and interesting study area. The location of the bay at the transition between the warm temperate south-coast biogeographic province and the cool temperate west-coast (Griffiths & Branch, 1991) has resulted in a species-rich mosaic of cold- and warm-water groups, although an analysis of benthic invertebrates by Day (1970) showed that the fauna of False Bay was more strongly dominated by species of the south-coast province.

Traditionally, the waters of False Bay have been, and remain important to both the local fishing industry and the South African Navy (van Ballengooyen, 1991), being utilised extensively for the harvesting of fish and shellfish by commercial and subsistence fishermen (Van der Merwe et al., 1991). It is also a major area for recreation and water sports, and its sandy and rocky beaches are visited by thousands of tourists every year (Taljaard et al., 2000). Part of its coastline also forms part of conservation areas. An investigation of the water quality within the bay is crucial to the development of long-term planning of pollution management strategies. It is therefore of major importance that pollution levels be monitored to provide information for the management of the area. Impact assessment studies in False Bay (Eagle, 1976; Brown, 1975; Bartlett & Hennig, 1982) have focused mainly on the effect of individual discharges on the immediate surroundings without considering cumulative impacts (Taljaard et al., 2000).

Previously, pollution in the bay was found to range from short-term microbial contamination, nutrient enrichment and organic matter contamination, to long-term trace metal accumulation, with a number of "hot-spots" being identified (Taljaard et al., 2000). Increasing concern about the pollution status of False Bay resulted in the monitoring of trace metals between 1985 and 1997 (Taljaard et al., 2000). The results showed that cadmium, lead and zinc were, on many occasions higher than the required limits, and that there were specific "hot spots" along the periphery of the bay. There is also a lack of information on the effects of pollution on the below-individual responses of invertebrates from the area, since previous studies focused mostly on the impact of pollutants on the beach whelk (Brown, 1982).

The main aim of the study was to provide baseline information on contamination levels and to evaluate suitable intertidal invertebrate species and biomarkers, which could be used as a basis for the design of assessment, monitoring and research programmes. The specific objectives of the present study were:

- To assess the extent of heavy metal pollution in the sediments and invertebrates in the False Bay intertidal zone.
- To identify cellular biomarkers that can serve as monitoring tools of environmental stress and possibly ecological impact of heavy metals.
- To determine the accumulation patterns of the heavy metal cadmium in the soft tissues of selected invertebrates.

To meet these objectives, sediment and water samples were collected from various sites in the False Bay intertidal zone, and the seasonal heavy metal concentrations (cadmium, copper, nickel, lead and zinc) were determined. The results obtained are discussed in Chapter 2, with the possible environmental parameters and anthropogenic sources that may contribute to the current levels. To assess the heavy metals in the invertebrates, samples of the barnacle *Tetraclita serrata,* the periwinkles *Oxystele tigrina* and *O. sinensis,* the limpet *Patella oculus* the mussel *Choromytilus meridionalis* and the starfish *Patiriella exigua* were collected seasonally, with the results from these assessments being discussed in Chapters 3-7 respectively.

Laboratory experiments were also carried out to determine the mechanisms of cadmium uptake, distribution and accumulation in the different organs, and elimination from the tissues of the different species (Chapter 8). Cadmium was chosen as a study metal because it is widely considered a priority contaminant in marine sediments and shows a wide variety of toxic effects at cellular and subcellular levels (Selck & Forbes, in press). The haemolymph lysosomal responses to environmental stress were investigated in field-collected organisms, as well as in laboratory-based cadmium exposure experiments (Chapter 9) using the Neutral Red Retention (NRR) assay and computer-assisted image analysis. The NRR assay was chosen because of the low cost, relatively short measuring time and the small sample size required (Riveros et al., 2002).

CHAPTER 2 – ENVIRONMENTAL PARAMETERS AND HEAVY METAL LEVELS IN THE WATER AND SEDIMENTS OF FALSE BAY

2.1. INTRODUCTION

Heavy metals are increasingly being introduced into the environment as contaminants and pollutants by processes such as atmospheric deposition of geological matrix (Goh & Chou, 1997), and through anthropogenic sources such as industrial effluents, mining wastes, wastewater effluents and auto emissions (Bu-Olayan & Subrahmanyam, 1998). Although many developed countries have implemented regulatory action to decrease metal contamination (McCally, 2002), environmental levels of metals in developing countries still remain high, mainly because of continued production and use of restricted toxic chemicals such as DDT and dioxin (UNEP, 2003), and the provision of sewage treatment facilities and municipal waste disposal which have not kept pace with global trends (GESAMP, 2001).

Heavy metals such as Cd, Cu, Ni, Pb and Zn tend to be elevated in the aquatic environment due to their persistent nature (Ankley et al., 1996). In the water column of contaminated surface waters, many of these pollutants tend to adsorb to suspended solids and eventually settle and become incorporated into sediments (Sarki et al., 1995). Although pollutants can remain biologically unavailable in the system, they can also be transformed into more or less toxic forms and become bioavailable (Carter & Porter, 1997).

The sediment milieu is a complex one, with numerous binding phases being responsible for regulating metal bioavailability (Sibley et al., 1999). In aquatic environments, heavy metal contamination is characterised by higher levels in the sediments and benthic organisms than in the water (Sarki et al., 1995), and accumulation can be up to three orders of magnitude higher than the aqueous phase (Deacon & Driver, 1999), particularly in organically-rich sediments (Rainbow, 1995).

Metal toxicity associated with sediments is of particular concern, since they can be released into the water column and adversely affect biota (Kang et al., 1999) and, because of their bio-accumulative nature, can result in toxicity to higher organisms (Ashley & Baker, 1999). The objective of this part of the study was to assess the extent of the heavy metal pollution in the False Bay intertidal zone by measuring the concentrations of these heavy metals in the surface water and sediments, and evaluating possible anthropogenic inputs.

2.2. DESCRIPTION OF THE STUDY AREA AND STUDY SITES

False Bay (Figure 1) is the largest true bay in South Africa (Heinecken et al., 1983). The bay is a roughly square body of water with a coastline of about 30 km, with a maximum depth of 90m at the south entrance (Day, 1970). The wide mouth of the bay permits free exchange of nutrients and chemicals with the open sea, with the residence time of water in the bay being approximately 4-6 days (Heinecken et al., 1983). Water is circulated clockwise from Muizenberg to Strand along the northern shoreline, and anti-clockwise at Gordon's Bay (CSIR, 1992). Depth increases from the northern margin to the mouth of the bay (Anon., 1980). A bi-directional wind regime prevails in False Bay, comprising of south-easterly winds in summer, and north-westerly winds in winter (Taljaard et al., 2000). The background levels (μg/g) for heavy metals in False Bay sediments were determined in previous studies as being: Cd = 0.05; Cu = 3; Ni = 4; Pb = 2.5 and Zn = 17 (DEAT, 1985).

Seven study sites along the False Bay coast were chosen for the present study. Site 1 (Strand) is situated in the Lourens River catchment, where land uses include residential developments, hotels and recreational sites, and light industries (Taljaard et al., 2000). The Lourens River opens into False Bay near to this study site. Site 2 (Gordon's Bay) is situated in the Sir Lowry's Pass catchment, where the land uses include formal housing, agriculture (vegetable farms and vineyards), a fishing-yacht harbour, holiday accommodation, a solid waste dumpsite and a wastewater treatment plant (Taljaard et al., 2000). The Sir Lowry's Pass River opens into the bay near this site.

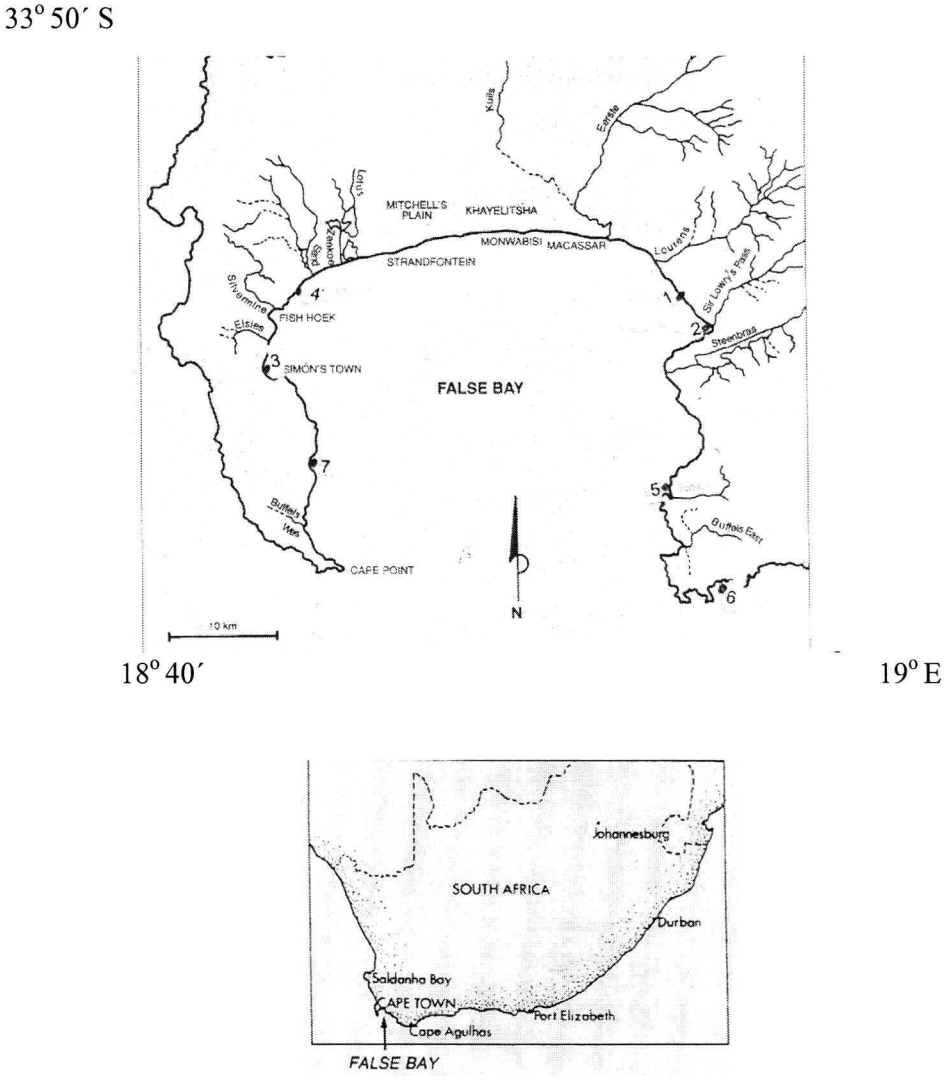

Figure 1: Map of South Africa showing position of False Bay, and map of False Bay, with numbers showing the positions of the different sampling stations (Taljaard et al., 2000). (1- Strand; 2- Gordon's Bay; 3- Glencairn; 4- Muizenberg; 5- Rooiels; 6- Kleinmond; 7- Miller's Point)

Sites 3 (Glencairn) and 7 (Miller's Point) both occur in the South Peninsula catchment (Taljaard et al., 2000) where land uses are limited to formal residential developments, a naval harbour north of site 3, and a railway line running close to the shore at site 3. A solid waste dumpsite, yacht club and landing strip for small aircraft occur near site 7 (Taljaard et al., 2000). The Elsies River drains into False Bay near site 3.

Site 4 (Muizenberg) is situated in the Muizenberg catchment, where the main developments include formal residences and a railway line running close to the shore (Taljaard et al., 2000). Both the Silvermine and Sand Rivers open near this study site. Site 5 (Rooiels) is situated within the Rooiels catchment, which opens into False Bay via the Rooiels River mouth (Taljaard et al., 2000). At this site, land use is limited to holiday residences and recreational facilities. The Rooiels catchment also drains an area of earlier military weapons testing activity (Cock & McKenzie, 1998). Site 6 (Kleinmond) occurs outside the eastern arm of False Bay, and is situated within the Hangklip/ Kleinmond catchment, where the main land uses include a fishing harbour, formal and informal residential developments (Taljaard et al., 2000). This site was chosen as a reference site, to compare the situation occurring within the semi-enclosed False Bay receiving various drainage outlets from a more highly populated area, with what occurs in the "open" sea, more remote from industrial and other anthropogenic influences.

The northern shore of the bay has the highest population of more than 1.6 million people, followed by the western arm, and the eastern arm being the least populated, except during the holiday periods (Quick, 1993). There is little development along the western arm from Cape Point to Simon's Town, and along the eastern arm between Gordon's Bay and Rooiels, while much of the urban development is found between Muizenberg and Gordon's Bay (Anon., 1980).

A number of point sources contribute to the pollution load of False Bay (CSIR, 1992). These include effluent discharge points from 14 sewage works (Anon., 1983), factory effluents at Simon's Town (near site 3) and Strand (site 1) (Eagle, 1976), herbicide and pesticide runoff from intensive farming, and storm water drainage from urban areas, with major outlets at Gordon's Bay (site 2), Muizenberg (site 4), Simon's Town

(near site 3) and Strand (site 1) (Heinecken et al., 1983). Land uses in False Bay include shore-based and water-based recreation, boating, diving, commercial fishery and bait collection, with harbour and shipping activities at Gordon's Bay and Kalk Bay, stormwater and wastewater discharges, and nature conservation (Quick, 1993).

Some 26% of the South African coast consists of mixed rock and sand shores (Brown et al., 1991), and about roughly the same proportion appears also in the False Bay shore. The northern shore between Muizenberg and Strand (Figure 1), is characterised by stretches of long sandy beach, broken only by few isolated outcrops of sandstone cliffs (Mallory, 1970), while the eastern and western shorelines are rocky and steep. The effect of False Bay's mixed shores is seen in the characteristic biota that they harbour, which is distinct from that of either rock or pure sand (Brown et al., 1991), and the unequal distribution of species in the different sampling sites, as seen in the absence of some species at some of the sampling sites.

There is a good deal of information on the sandy beach fauna of False Bay, such as *Bullia* species, but very little on the rocky shore species (Stenton-Dozey & Brown, 1991). Previous studies have also focused on the impact of pollutants on the beach whelk (Brown, 1982) but none on the species under investigation in the present study, while some studies (Brown, 1975; Eagle, 1976; Bartlett & Hennig, 1982) have looked at the effect of individual outfalls on the surrounding environment.

2.3. MATERIALS AND METHODS

2.3.1. Sediment sampling

Sediment samples were collected seasonally between July 2000 and July 2001, during the months of July 2000 (winter), September 2000 (spring), December 2000 (summer), April 2001 (autumn) and July 2001 (winter). A seasonal sampling approach was chosen in order to determine whether any temporal variations in heavy metal levels would be observed. The samples were collected at low tide. Sampling was carried out at the six sites within False Bay, and the reference site (site 6) situated

outside the bay. The sites were chosen based on land uses in the catchments and their potential to contribute towards pollution.

The sediment samples were collected using a small plastic hand scoop ($0.1m^2$). Twenty replicate samples were collected at each site at regular intervals of 10m for up to 500m, put in plastic buckets and immediately placed on ice in cooler boxes for transporting to the laboratory. In the laboratory, the samples from each site were homogenized with a stainless steel blender and then oven-dried, and then particle size was determined on 50 g of each sample using the dry sieving method by Briggs (1981).

2.3.2. Water sampling

Water samples were collected simultaneously from the same area as the sediments at low tide, at 30cm depth above the sediments and about 10-15 m from the shore. Since the sediment-water interface of a marine basin is an area where the greatest gradients in chemical and physical properties occur, and which can in turn affect metal concentrations (Zago et al., 2000), the water temperature was measured in the field using a thermometer. The samples were then placed in pre-cleaned 5-litre plastic containers and transported to the laboratory, where the pH and water salinity were determined using a pH meter (Crison micro pH 2001 model) and salinometer respectively. A litre of each of the water samples was filtered through a 0.45 um Whatman membrane filter, acidified with 1ml of concentrated nitric acid per litre of water, and then frozen until further analysis.

2.3.3. Rainfall data

The rainfall data for False Bay, collected at two weather stations from March to October 2000, and from March to October 2001, was obtained from the South African Weather Bureau.

2.3.4. Heavy metal analyses

Heavy metal analyses were carried out in the Department of Physics of the University of Stellenbosch. For this purpose, the frozen samples were defrosted, and the sediment samples were oven-dried at 60^0C for 48h. Aliquot samples (0.2-0.5 g for sediment and 5 ml for water) were digested with 10 ml of nitric acid and left to stand overnight. The next day, samples were heated at 40- 60^0 for 2h in a test tube heating block, and then for a further 1h at 110-120^0 C. The digestates were allowed to cool before adding 1 ml of perchloric acid and re-heating at 110-120^0 C until brown fumes appeared. The digestates were allowed to cool again. Distilled water (5 ml) was added to the digestates, which were heated again until white fumes appeared. The digestates were left to cool overnight and then double-filtered through a Whatman No. 6 filter paper and a 0.45 membrane micro-filter paper.

A blank accompanied each batch of samples through all analytical steps. The concentrations of cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) were analyzed, with five analyses done for each sample, using the flame atomic absorption spectrophotometer (Varian AA-1275) with acetylene-air flame, and the inductively coupled plasma atomic emission spectrophotometer (ICP, HP 4500 model). Concentrations of heavy metals are given as μg/g dry weight. Accuracy and efficiency of the extraction methods were made by adding known amounts of representative metals to aliquots of sample tissues from each species and determining the recovery of each metal. Percentage recoveries were between 90 and 95%. Analytical standards were used for all the metals to determine the analytical limits, which were found to be 0.02 for Cd and Zn; 0.1 for Cu; 0.03 for Ni and 0.05 for Pb.

2.3.5. Statistical analysis

All calculations were done with the Jandel Scientific Sigmastat 2.0 program. One-way ANOVA was used to determine whether there were any seasonal and spatial differences in the means heavy metal concentrations, while t-tests were used to determine significant differences between sediment and water concentrations.

The Spearman Rank correlation analyses were carried out to determine the relationships between physical parameters (pH, temperature and salinity) and heavy metal concentrations. The relationships among the heavy metals in water, and among those in sediments were also determined using the Spearman Rank correlation analyses. The Pearson Product Moment correlation analyses were used to determine the relationship between heavy metals in the water and sediments of each site during the different seasons.

To quantify the magnitude of pollution by the different heavy metals, the contamination factor (CF), which indicates by which factor the background concentration is exceeded at a site, was calculated using the equation:

CF = Metal concentration in sediments/ background levels for shallow marine sediments (El-Sammak & Aboul-Kassim, 1999).

The Pollution Load Index (PLI), which is used to determine mutual pollution effect at different sites by different metals (El-Sammak & Aboul-Kassim, 1999), was then calculated using the equation: $PLI = \sqrt[7]{(CF_{Cd})\ (CF_{Cu})\ (CF_{Ni})\ (CF_{Pb})\ (CF_{Zn})}$. According to El-Sammak & Aboul-Kassim (1999), a higher value of the pollution index indicates increased pollution.

2.4. RESULTS

2.4.1. Sediment parameters

2.4.1.1. *Particle size*

The mean sediment particle size ranged between medium (0.25 mm) and coarse (2 mm) (Table 1), with the sediments from sites 4 and 6 having smaller-particle sediments compared to the other sites.

TABLE 1: Ranges of the sediment mean particle sizes measured from the different sites (Figure 1) (Site 1- Strand; 2- Gordon's Bay; 3- Glencairn; 4- Muizenberg; 5- Rooiels; 6- Kleinmond; 7- Miller's Point)

Sites	Sediment grain size (mm)
1	0.5 – 1.00
2	0.5 – 2.00
3	0.5 – 1.00
4	0.25 –1.00
5	1.00 – 2.00
6	0.25 – 0.50
7	1.00 – 2.00

2.4.1.2. Heavy metal concentrations in sediments

2.4.1.2.1. Cadmium

Table 2 shows the Cd concentrations measured in the sediments at the different sites during the various seasons. The mean Cd concentrations ranged between undetectable levels and 12.36 (± 0.21) µg/g. The values tended to decrease from winter to spring, and then increased during summer, through autumn to winter 2001. One-way ANOVA showed that the sediments from site 4 had significantly higher Cd concentrations compared to the other sites ($p < 0.001$, $n = 5$ pool of 20 samples). There were significant seasonal differences, with concentrations in the samples collected during winter 2000 being significantly higher ($p < 0.001$, $n = 5$) than those obtained during the other seasons at sites 1, 4, 5 and 6. The winter 2001 samples had significantly higher concentrations than those of other seasons at sites 2, 3 and 7 ($p < 0.001$, $n = 5$).

Table 2: Mean Cd concentrations (µg/g ± SD) measured in sediment samples from the different sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	7.36 ± 0.21	1.54 ± 0.02	3.0 ± 0.04	3.09 ± 0.01	5.16 ± 0.25
Site 2	3.03 ± 0.10	1.01 ± 0.01	5.25 ± 0.2	6.17 ± 0.01	6.9 ± 0.01
Site 3	0.21 ± 0.02	0.1 ± 0.02	ND	0.21 ± 0.01	1.21 ± 0.22
Site 4	12.36 ± 0.21	2.77 ± 0.03	10.75 ± 0.02	10.46 ± 0.01	11.79 ± 0.28
Site 5	7.0 ± 0.03	0.32 ± 0.01	0.1 ± 0.02	1.25 ± 0.02	3.39 ± 0.25
Site 6	2.8 ± 0.29	0.61 ± 0.02	1.0 ± 0.04	1.75 ± 0.02	2.1 ± 0.25
Site 7	0. 2 ± 0.01	0.30 ± 0.01	1.3 ± 0.01	1.59 ± 0.01	1.77 ± 0.02

* ND- not detected

2.4.1.2.2. Copper

For Cu, the mean concentrations in the sediments (Table 3) ranged between undetectable levels and 15.10 (± 0.01) µg/g. The values decreased from winter to summer; being undetected at all other sites except site 7 during summer, and then increased from autumn to winter 2001. One-way ANOVA showed that these seasonal variations were significantly different ($p < 0.001$, $n = 5$ pool of 20 samples). There were also significant spatial differences ($p < 0.001$, $n = 25$), with site 1 concentrations being significantly higher than those from the other sites during winter 2000, site 5 concentrations higher during spring, and site 7 concentrations being significantly higher from summer, through autumn to winter 2001 ($p < 0.001$, $n = 5$).

Table 3: Mean Cu concentrations (µg/g ± SD) measured in sediment samples from the different sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	15.10 ± 0.01	ND	ND	1.24 ± 0.01	6.77 ± 0.01
Site 2	3.18 ± 0.02	ND	ND	0.98 ± 0.01	0.98 ± 0.01
Site 3	1.61 ± 0.01	ND	ND	6.05 ± 0.02	6.05 ± 0.02
Site 4	5.27 ± 0.01	1.41 ± 0.01	ND	7.46 ± 0.01	7.46 ± 0.01
Site 5	ND	4.70 ± 0.01	ND	3.50 ± 0.02	3.50 ± 0.02
Site 6	2.16 ± 0.01	0.30 ± 0.01	ND	2.22 ± 0.02	2.22 ± 0.02
Site 7	11.0 ± 0.03	2.71 ± 0.01	1.94 ± 0.01	11.50 ± 0.02	11.50 ± 0.02

* ND- not detected

2.4.1.2.3. Nickel

Ni concentrations of the sediments during the various seasons are shown in Table 4. The values ranged between 6.42 (± 0.17) and 50.00 (± 0.40) µg/g. One-way ANOVA showed that concentrations differed significantly both seasonally and spatially ($p < 0.05$, $n = 5$ pool of 20 samples). The concentrations of samples from site 5 (Appendix 3) were significantly higher than those from the other sites during winter 2000, those from site 1 were higher during spring, those from site 4 were higher during summer and winter 2001, and those from site 2 were significantly higher during autumn. The mean concentrations tended to decrease from winter to spring, and then increased from summer, through autumn to winter 2001.

Table 4: Mean Ni concentrations (µg/g ± SD) measured in sediments from different sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	12.21 ± 0.03	17.50 ± 0.03	22.50 ± 0.25	30.29 ± 0.33	32.99 ± 0.44
Site 2	24.24± 0.27	16.90 ± 0.31	30.25 ± 0.12	37.27 ± 0.16	38.01 ± 0.18
Site 3	6.42 ± 0.17	0.52 ± 0.02	13.04 ± 0.24	16.71 ± 0.26	19.91 ± 0.13
Site 4	18.18 ± 0.10	9.40 ± 0.21	50.0 ± 0.38	30.86 ± 0.02	46.82 ± 0.01
Site 5	37.17 ± 0.10	3.18 ± 0.13	10.0 ± 0.10	14.33 ± 0.03	34.33 ± 0.18
Site 6	29.22 ± 0.10	12.58 ± 0.13	8.76 ± 0.02	13.79 ± 0.10	23.94 ± 0.35
Site 7	29.50 ± 0.15	15.79 ± 0.20	9.63 ± 0.10	11.59 ± 0.20	31.50 ± 0.03

2.4.1.2.4. Lead

Table 5 shows the concentrations of Pb measured in the sediments at the different sites during the different seasons. The mean concentrations ranged between 2.00 (± 0.02) and 60.76 (± 0.10) µg/g, which were measured at site 1 during spring, and at site 2 during winter 2000. One-way ANOVA showed that there were significant seasonal and spatial variations in Pb concentrations ($p > 0.05$, $n = 5$). During winter 2000, the mean concentrations were in the order: site 2 > site 7 > site 6 > site 4 > site 1 > site 3 > site 5. During spring, the concentrations were in the order: site 4 > site 2> site 6> site 7> site 5> site 3> site 1. During summer, the concentrations were in the order: site 2> site 3> site 4> site 7> site 6> site 5> site 1. The concentrations measured during autumn were in the order: site 4> site 2> site 3> site 7> site 6> site 1> site 5.

Table 5: Mean Pb concentrations (µg/g ± SD) in sediment samples from the different sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels;6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	15.15 ± 0.10	2.0 ± 0.02	10.08 ± 0.03	16.80 ± 0.10	17.0 ± 0.10
Site 2	60.76± 0.10	38.46 ± 0.04	35.08 ± 0.04	36.06 ± 0.02	40.49 ± 0.16
Site 3	10.84 ± 0.10	3.68 ± 0.04	30.08 ± 0.02	34.65 ± 0.02	34.74 ± 0.02
Site 4	28.41 ± 0.02	47.28 ± 0.10	28.64 ± 0.10	37.33 ± 0.10	41.50 ± 0.14
Site 5	2.33 ± 0.13	8.49 ± 0.04	11.0 ± 0.04	15.0 ± 0.10	15.85 ± 0.10
Site 6	32.43 ± 0.02	30.30 ± 0.04	20.0 ± 0.04	27.84 ± 0.04	30.05 ± 0.02
Site 7	41.0 ± 0.04	22.90 ± 0.10	23.33 ± 0.02	30.70 ± 0.04	41.50 ± 0.02

2.4.1.2.5. Zinc

For Zn, the mean concentrations in the sediments ranged between 14.10 (± 0.04) and 119.55 (± 0.34) µg/g, with the lowest being measured at site 3 and the highest at site 4 (Table 6). At most sites, the values decreased from winter to spring 2000, then increased from summer through autumn to winter 2001. One-way ANOVA showed that these spatial and seasonal differences were highly significant ($p < 0.05$, $n = 5$ pool of 20 samples).

Table 6: Mean Zn concentrations (µg/g ± SD) in sediment samples from the different sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	38.24 ± 0.02	21.60 ± 0.02	81.25 ± 0.10	85.91 ± 0.01	90.25 ± 0.01
Site 2	72.71± 0.03	30.77 ± 0.03	56.20 ± 0.10	67.37 ± 0.04	69.44 ± 0.10
Site 3	14.10 ± 0.04	40.0 ± 0.10	65.0 ± 0.10	20.98 ± 0.10	34.83 ± 0.03
Site 4	26.73 ± 0.10	21.54 ± 0.10	100.0 ± 0.03	110.46 ± 0.10	119.55 ± 0.02
Site 5	100 ± 0.34	90.91 ± 0.29	41.50 ± 0.10	67.67 ± 0.03	80.27 ± 0.04
Site 6	59.23 ± 0.02	20.0 ± 0.04	72.73 ± 0.23	77.83 ± 0.10	80.49 ± 0.10
Site 7	67.50 ± 0.03	39.36 ± 0.04	46.57 ± 0.10	61.50 ± 0.04	71.75 ± 0.10

2.4.2. Water parameters

2.4.2.1. *Temperature*

Table 7 shows the mean water temperatures measured at the sites during the different seasons. There was a rapid decrease in the mean temperatures from summer to autumn. A local upwelling of cold water caused lower temperatures at site 7. One-way ANOVA showed that there were significant seasonal differences in the mean temperatures ($p < 0.001$, $n = 5$ pool of 20 samples).

Table 7: Mean water temperatures (°C ± SE) measured at the different sites during five seasons(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	14.0 ± 0.12	18.02 ± 0.10	24.11 ± 0.10	17.16 ± 0.10	15.0 ± 0.11
Site 2	14.1 ± 0.13	18.1 ± 0.12	25.05 ± 0.11	18.0 ± 0.10	15.12 ± 0.12
Site 3	14.4 ± 0.10	17.05 ± 0.12	19.1 ± 0.11	15.17 ± 0.10	14.2 ± 0.10
Site 4	14.12 ± 0.10	18.11 ± 0.10	19.2 ± 0.12	15.05 ± 0.10	14.7 ± 0.10
Site 5	14.8 ± 0.12	18.15 ± 0.12	23.03 ± 0.10	14.09 ± 0.10	14.9 ± 0.12
Site 6	14.1 ± 0.04	18.17 ± 0.10	22.06 ± 0.04	16.1 ± 0.04	16.0 ± 0.10
Site 7	13.3 ± 0.10	16.01 ± 0.11	18.4 ± 0.10	14.14 ± 0.10	15.1 ± 0.12

2.4.2.2. *pH*

The water pH (Table 8) ranged between 7.10 (± 0.04) and 8.67 (± 0.03). One-way ANOVA showed that the spring and summer values were significantly higher than those measured during winter and autumn ($p < 0.001$, $n = 5$ pool of 20 samples).

Table 8: Mean water pH (± SE) measured at the different sites during five seasons(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	7.2 ± 0.12	8.06 ± 0.10	8.17 ± 0.10	7.49 ± 0.10	7.3 ± 0.10
Site 2	7.15 ± 0.11	8.14 ± 0.10	8.23 ± 0.10	7.77 ± 0.10	7.45 ± 0.12
Site 3	7.53 ± 0.10	8.12 ± 0.10	8.27 ± 0.12	7.36 ± 0.13	7.1 ± 0.12
Site 4	7.2 ± 0.11	7.93 ± 0.10	8.67 ± 0.10	7.29 ± 0.10	7.22 ± 0.10
Site 5	7.1 ± 0.10	8.32 ± 0.10	8.2 ± 0.10	7.41 ± 0.10	7.3 ± 0.10
Site 6	7.2 ± 0.10	7.15 ± 0.12	7.35 ± 0.10	7.6 ± 0.11	7.8 ± 0.10
Site 7	7.30 ± 0.11	8.01 ± 0.10	8.31 ± 0.10	7.3 ± 0.12	7.4 ± 0.10

2.4.2.3. *Salinity*

Table 9 shows the water salinities measured at the different sampling stations. The lowest values measured during all five seasons were recorded at site 1. During winter 2000, salinity ranged between 29.24 (± 0.03) and 35.00 (± 0.10) $^{0}/_{00}$. During spring, the values increased slightly to values between 30.10 (± 0.10) and 35.16 (± 0.10) $^{0}/_{00}$. The summer values ranged between 31.00 (± 0.04) and 35.24 (± 0.03) $^{0}/_{00}$ while those measured during autumn ranged between 30.16 (± 0.03) and 35.18 (± 0.03) $^{0}/_{00}$. During winter 2001, the salinities decreased again to values ranging between 28.40 (± 0.04) and 35.10 (± 0.10) $^{0}/_{00}$, with the highest being measured at site 2 and the lowest measured at site 1. One-way ANOVA showed that while significant seasonal differences in salinity occurred ($p < 0.001$, $n = 5$), there were, however, no significant spatial differences ($p > 0.05$, $n = 5$).

TABLE 9: Mean water salinities ($^0/_{00}$) (± SE) measured at the different sites during five seasons (n = 5 pool of 20 samples)

Sites	Winter '00	Spring	Summer	Autumn	Winter '01
1	29.24 ± 0.30	30.10 ± 0.03	31.00 ± 0.04	30.16 ± 0.03	28.40 ± 0.04
2	35.00 ± 0.10	35.10 ± 0.10	35.20 ± 0.10	35.15 ± 0.04	35.10 ± 0.10
3	35.04 ± 0.04	35.12 ± 0.04	35.20 ± 0.04	35.18 ± 0.03	35.07 ± 0.03
4	35.00 ± 0.10	35.16 ± 0.10	35.22 ± 0.03	35.17 ± 0.10	35.00 ± 0.04
5	33.00 ± 0.10	34.00 ± 0.10	34.15 ± 0.10	34.08 ± 0.04	34.00 ± 0.10
6	34.20 ± 0.04	34.05 ± 0.10	34.01 ± 0.10	35.00 ± 0.10	34.00 ± 0.20
7	35.00 ± 0.03	35.15 ± 0.10	35.24 ± 0.03	35.16 ± 0.03	35.00 ± 0.10

2.4.3. Rainfall data measured in False Bay during the study period

The rainfall data measured at two weather stations situated within the study area was obtained (SAWB, 2002), and is shown in Table 10. There were significant differences between the rainfall amounts recorded during 2000 and 2001 ($p < 0.001$, $n = 8$), with significantly higher levels occurring during the year 2001. Significantly higher rainfall was recorded during the month of July 2001 at both stations ($p < 0.001$, $n = 8$).

TABLE 10: Monthly mean rainfall (mm) recorded at two weather stations situated in the False Bay area during the years 2000 and 2001

Month	Strand		Simon'stown/ Cape Point	
	2000	2001	2000	2001
March	15.8	1.2	22.8	0.4
April	8.0	38.2	11.2	26.8
May	85.6	136.2	16.2	49.0
June	79.6	44.8	30.6	41.4
July	77.8	197.6	25.4	106.4
August	86.0	93.6	23.4	61.2
September	74.0	63.6	36.4	28.8
October	5.0	55.6	2.4	21.8

2.4.4. Heavy metal concentrations of water

2.4.4.1. Cadmium

Cd concentrations measured in the water samples are shown in Table 11. The mean concentrations at most sites decreased from winter to spring, and then increased from summer, through autumn to winter 2001. The mean concentrations of the samples obtained during winter 2001 were significantly higher than those obtained at the other times of the year ($p < 0.001$, $n = 5$ pool of 20 samples). There were also significant spatial differences ($p < 0.001$, $n = 5$), with the highest mean Cd concentration being measured in the water samples from site 5 during winter 2000.

Table 11: Mean Cd concentrations in water samples (µg/L ± SE, n = 5) from the different sites during five seasons (Site 1- Strand; 2- Gordon's Bay; 3- Glencairn; 4- Muizenberg; 5-Rooiels; 6-Kelinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	0.15 ± 0.02	0.20 ± 0.02	1.05 ± 0.03	2.0 ± 0.10	2.49 ± 0.10
Site 2	0.65 ± 0.03	0.04 ± 0.01	1.0 ± 0.02	1.94 ± 0.04	2.47 ± 0.10
Site 3	0.05 ± 0.01	0.15 ± 0.02	3.24 ± 0.10	6.60 ± 0.10	6.94 ± 0.04
Site 4	0.75 ± 0.04	0.55 ± 0.03	1.37 ± 0.10	2.01 ± 0.02	2.40 ± 0.02
Site 5	10.40 ± 0.10	0.19 ± 0.02	0.10± 0.02	0.97 ± 0.04	1.77 ± 0.04
Site 6	0.19 ± 0.03	0.40 ± 0.03	0.60 ± 0.02	1.20 ± 0.03	2.09 ± 0.02
Site 7	ND	ND	0.15 ± 0.02	1.49 ± 0.02	1.88 ± 0.02

*ND- not detected

2.4.4.2. Copper

The highest mean Cu concentration was measured in samples from site 5 (Table 12). The mean concentrations decreased from winter to spring 2000, and were undetected at site 1 during winter 2000 and spring, at sites 2 and 3 during, at site 5 during summer, at site 6 during winter 2000, and at site 7 during winter 2000 and spring. The mean concentrations increased again during summer, through autumn to winter 2001, especially at sites 1, 2, 4 and 6. One-way ANOVA showed that these seasonal and spatial mean concentrations were significantly different ($p < 0.05$, $n = 5$).

Table 12: Mean Cu concentrations in water samples (µg/L ± SE, n = 5) from the different sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	ND	ND	0.67± 0.02	1.10 ± 0.03	2.09 ± 0.10
Site 2	1.55 ± 0.02	ND	0.67± 0.02	0.95 ± 0.04	1.15 ± 0.02
Site 3	2.20 ± 0.02	ND	0.12 ± 0.03	1.84 ± 0.03	2.30 ± 0.03
Site 4	0.60 ± 0.04	0.60 ± 0.02	0.40 ± 0.03	1.22 ± 0.01	3.10 ± 0.02
Site 5	4.65 ± 0.02	1.05 ± 0.03	ND	1.04 ± 0.02	3.06 ± 0.02
Site 6	ND	2.70 ± 0.03	1.05 ± 0.02	1.99 ± 0.04	2.24 ± 0.03
Site 7	ND	ND	ND	2.30 ± 0.02	2.45 ± 0.03

*ND- not detected

2.4.4.3. Nickel

Ni (Table 13) was undetected during winter 2000 in the water samples obtained from all the other sites except those from sites 3 and 4. During spring, Ni was detected only at site 4. The mean concentrations increased at most sites during summer, through to autumn, until they reached the significantly higher levels recorded during winter 2001. ANOVA showed that these seasonal and spatial differences were highly significant ($p < 0.001$, n = 5 pool of 20 samples).

Table 13: Mean Ni concentrations in water samples (µg/L ± SE, n = 5) from the different sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	ND	ND	ND	1.10 ± 0.04	2.05 ± 0.02
Site 2	ND	ND	2.20± 0.02	3.45 ± 0.02	6.35 ± 0.04
Site 3	1.52 ± 0.10	ND	5.20 ± 0.10	6.49 ± 0.03	8.30 ± 0.02
Site 4	0.15 ± 0.02	0.50 ± 0.02	5.08 ± 0.03	6.30 ± 0.02	10.74 ± 0.02
Site 5	ND	ND	3.72 ± 0.03	3.99 ± 0.02	7.35 ± 0.02
Site 6	ND	ND	3.60 ± 0.03	4.0 ± 0.10	6.20 ± 0.02
Site 7	ND	ND	ND	0.15 ± 0.02	1.30 ± 0.03

2.4.4.4. Lead

Table 14 shows the Pb concentrations obtained in the water samples from the various sites. Significant seasonal variations occurred ($p < 0.001$, n = 5 pool of 20 samples), with concentrations increasing from winter 2000 to winter 2001 at most sites. The lowest mean concentration (0.04 ± 0.02 µg/L) was obtained in samples from site 7 during spring, and the highest (14.00 ± 0.10 µg/L) from site 1 during winter 2001.

Table 14: Mean Pb concentrations in water samples (µg/L ± SE, n = 5) from the different sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	0.14 ± 0.03	0.15 ± 0.02	1.70 ± 0.02	2.20 ± 0.02	14.0 ± 0.10
Site 2	0.50 ± 0.04	0.35 ± 0.01	1.05 ± 0.02	2.84 ± 0.02	6.30 ± 0.02
Site 3	1.15 ± 0.02	0.35 ± 0.02	4.80 ± 0.03	6.30 ± 0.02	9.20 ± 0.01
Site 4	0.30 ± 0.02	10.17 ± 0.04	5.20 ± 0.03	4.30 ± 0.10	11.40 ± 0.02
Site 5	1.65 ± 0.02	0.60 ± 0.02	4.65 ± 0.02	5.09 ± 0.03	10.30 ± 0.03
Site 6	0.30 ± 0.02	0.30 ± 0.03	3.05 ± 0.02	3.85 ± 0.02	4.90 ± 0.03
Site 7	0.67 ± 0.01	0.04 ± 0.02	0.35 ± 0.02	3.40 ± 0.03	5.20 ± 0.03

2.4.4.5. Zinc

There were significant spatial variations in the Zn concentrations ($p < 0.001$, $n = 5$) (Table 15). The highest mean concentration (48.05 ± 0.10 µg/L) was obtained in the samples from site 5, while the lowest (1.90 ± 0.02 µg/L) was measured at site 7. There were also significant seasonal differences ($p < 0.05$, $n = 5$ pool of 20 samples), with the mean concentration obtained in the samples collected during winter 2000 being significantly higher than those obtained during the other seasons at sites 2, 3, 4 and 5, while the winter 2001 concentrations were significantly higher than those of the other seasons at sites 1, 6 and 7 ($p < 0.001$, $n = 5$).

Table 15: Mean Zn concentrations in water samples (µg/L ± SE, n = 5) from the different sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	3.35 ± 0.02	4.30 ± 0.03	9.95 ± 0.03	10.12 ± 0.04	12.44 ± 0.02
Site 2	16.95± 0.02	12.30 ± 0.03	8.0 ± 0.10	10.95 ± 0.03	14.87± 0.02
Site 3	24.60 ± 0.03	8.10 ± 0.02	16.3 ± 0.03	17.80 ± 0.04	20.30 ± 0.02
Site 4	30.15± 0.03	9.30 ± 0.02	16.65 ± 0.10	17.0 ± 0.04	18.22 ± 0.02
Site 5	48.05 ± 0.10	5.60 ± 0.02	5.13 ± 0.03	7.09 ± 0.03	10.50 ± 0.01
Site 6	4.35 ± 0.02	8.85 ± 0.04	7.10 ± 0.02	9.40 ± 0.02	14.60 ± 0.10
Site 7	4.50 ± 0.02	1.90 ± 0.02	3.55 ± 0.10	6.30 ± 0.03	11.97 ± 0.01

2.4.5. Comparisons between water and sediment concentrations of different heavy metals

2.4.5.1. Cadmium concentrations

The comparisons of the water and sediment concentrations of each heavy metal at each site were carried out using t-tests. For Cd, the water concentrations increased gradually from winter 2000 to the following winter, while the sediment concentrations decreased from winter to spring, and then increased again from summer through autumn to winter 2001 (Table 16). The sediment concentrations were significantly higher than those of the water samples during all five seasons at sites 1, 2 and 4

($p < 0.05$, $n = 5$). At site 3, there was no significant difference in the mean concentrations of Cd measured in the sediment and the water samples during spring ($p > 0.05$, $n = 5$), while the water concentrations at this site were significantly higher than those of the sediment samples during summer, autumn and winter 2001 ($p < 0.00$, $n = 5$).

Table 16: A comparison of Cd concentrations in water (µg/L) and sediments (µg/g) (± SE, n = 5) from the sites where significant differences were observed (Site 1- Strand; site 2- Gordon's Bay; site 4- Muizenberg; site 7- Miller's Point)

	Winter 2000	Spring	Summer	Autumn 2001	Winter 2001
	Site 1				
Sediment (µg/g)	7.36 ± 0.21	1.54 ± 0.02	3.0 ± 0.04	3.09 ± 0.01	5.16 ± 0.25
Water (µg/L)	0.15 ± 0.02	0.20 ± 0.02	1.05 ± 0.03	2.0 ± 0.10	2.49 ± 0.10
	Site 2				
Sediment (µg/g)	3.03 ± 0.10	1.01 ± 0.01	5.25 ± 0.20	6.17 ± 0.01	6.9 ± 0.01
Water (µg/L)	0.65 ± 0.03	0.04 ± 0.01	1.0 ± 0.02	1.94 ± 0.04	2.47 ± 0.10
	Site 4				
Sediment (µg/g)	12.36 ± 0.21	2.77 ± 0.03	10.75 ± 0.02	10.46 ± 0.01	11.79 ± 0.28
Water (µg/L)	0.75 ± 0.04	0.55 ± 0.03	1.37 ± 0.10	2.01 ± 0.02	2.4 ± 0.02
	Site 7				
Sediment (µg/g)	11.0 ± 0.03	2.71 ± 0.01	1.94 ± 0.01	11.5 ± 0.02	12.0 ± 0.10
Water (µg/L)	ND	ND	ND	2.3 ± 0.02	2.45 ± 0.03

* **ND-** not detected

2.4.5.2. Copper concentrations

For Cu concentrations, significant differences were obtained during all five seasons in the samples from site 7 ($p < 0.05$, $n = 5$) (Table 17). Cu was not detected in the water samples from this site from winter 2000 to summer. At sites 1 and 6, the mean Cu concentrations in the sediments were significantly higher than those of the water concentrations during winter 2000, autumn and winter 2001 ($p < 0.001$, $n = 5$).

At site 2, the sediment concentrations were significantly higher than the water concentrations during the two winter periods ($p < 0.001$, $n = 5$).

Table 17: A comparison of Cu concentrations in water (µg/L) and sediments (µg/g) (± SE, n = 5) from the site where significant differences were observed (Site 7- Miller's Point)

	Winter 2000	Spring	Summer	Autumn 2001	Winter 2001
	Site 1				
Sediment (µg/g)	11.0 ± 0.03	2.71 ± 0.01	1.94 ± 0.01	11.50 ± 0.02	12.0 ± 0.10
Water (µg/L)	ND	ND	ND	2.3 ± 0.02	2.45 ± 0.03

*ND- not detected

2.4.5.3. Nickel

There were significant differences between the water and sediments concentrations of Ni during all the five seasons, at sites 1, 2, 4, 6 and 7 ($p < 0.05$, $n = 5$) (Table 18). At site 1, Ni was not detected in the water samples until autumn, while at site 2, Ni was undetected in the water samples during winter and spring. At the reference site (site 6), there was significant difference in the mean Ni concentration, with Ni being undetected in the water samples until summer, and the highest mean concentration being obtained during winter 2001. The highest mean Ni concentration in the sediments at this site was measured during winter 2001. Ni concentrations were below detection levels in the water samples from site 7 from winter 2000 until autumn 2001.

Table 18: A comparison of Ni concentrations in water (µg/L) and sediments (µg/g) (± SE, n = 5) from the sites where significant differences were observed (Site 1- Strand; site 2- Gordon's Bay; site 4- Muizenberg; site 6- Kleinmond; site 7- Miller's Point)

	Winter 2000	Spring	Summer	Autumn 2001	Winter 2001
	Site 1				
Sediment (µg/g)	12.21 ± 0.03	17.5 ± 0.03	22.5 ± 0.25	30.29 ± 0.33	32.99 ± 0.44
Water (µg/L)	ND	ND	ND	1.10 ± 0.04	2.05 ± 0.02
	Site 2				
Sediment (µg/g)	24.24 ± 0.27	16.9 ± 0.31	30.25 ± 0.12	37.27 ± 0.16	38.01 ± 0.18
Water (µg/L)	ND	ND	2.2 ± 0.02	3.45 ± 0.02	6.35 ± 0.04
	Site 4				
Sediment (µg/g)	18.18 ± 0.10	9.40 ± 0.21	50.0 ± 0.38	30.86 ± 0.02	46.82 ± 0.01
Water (µg/L)	0.15 ± 0.02	0.50 ± 0.02	5.08 ± 0.03	6.3 ± 0.02	10.74 ± 0.02
	Site 6				
Sediment (µg/g)	29.22 ± 0.10	12.58 ± 0.13	8.76 ± 0.02	13.79 ± 0.10	23.94 ± 0.35
Water (µg/L)	ND	ND	3.6 ± 0.03	4.0 ± 0.10	6.2 ± 0.02
	Site 7				
Sediment (µg/g)	29.5 ± 0.15	15.79 ± 0.20	9.63 ± 0.10	11.59 ± 0.20	31.5 ± 0.03
Water (µg/L)	ND	ND	ND	0.15 ± 0.02	1.30 ± 0.03

2.4.5.4. Lead

The t-tests showed that Pb concentrations in the sediment samples were significantly higher compared to those in water samples during all five seasons, at all the sites ($p < 0.05$, $n = 5$) (Table 19**).** At site 3, the mean Pb concentrations in both the water and sediments decreased from winter to spring, then increased again from summer through to winter 2001. The mean Pb concentrations in the water samples from site 4 increased from winter 2000 to spring, decreased from summer to autumn, and then increased again during winter 2001. The sediment concentrations from this site increased from winter to spring, decreased during summer, then increased again from autumn to winter 2001. The Pb concentrations in the water samples from the reference site (site 6) were similar during winter and spring, increasing gradually from summer,

through autumn to winter 2001. At site 7, both the water and sediment samples decreased from winter to spring, and then increased again from summer, through autumn to winter 2001.

Table 19: A comparison of Pb concentrations in water (µg/L) and sediments (µg/g) (± SE, n = 5) from the sites where significant differences were observed (Site 1- Strand; site 2- Gordon's Bay; site 3- Glencairn; site 4- Muizenberg; site 5- Rooiels; site 6- Kleinmond; site 7- Miller's Point)

	Winter 2000	Spring	Summer	Autumn 2001	Winter 2001
	Site 1				
Sediment (µg/g)	15.15 ± 0.10	2.9 ± 0.02	10.08 ± 0.03	16.8 ± 0.10	17.0 ± 0.10
Water (µg/L)	0.14 ± 0.03	0.15 ± 0.02	1.7 ± 0.02	2.2 ± 0.02	14.0 ± 0.10
	Site 2				
Sediment (µg/g)	60.76 ± 0.10	38.46 ± 0.04	35.08 ± 0.04	36.06 ± 0.02	40.49 ± 0.16
Water (µg/L)	0.5 ± 0.04	0.35 ± 0.01	1.05 ± 0.02	2.84 ± 0.02	6.30 ± 0.02
	Site 3				
Sediment (µg/g)	10.84 ± 0.10	3.68 ± 0.04	30.08 ± 0.02	34.65 ± 0.02	34.74 ± 0.02
Water (µg/L)	1.15 ± 0.02	0.35 ± 0.02	4.8 ± 0.02	6.3 ± 0.02	9.2 ± 0.01
	Site 4				
Sediment (µg/g)	28.41 ± 0.02	47.28 ± 0.10	28.64 ± 0.10	37.33 ± 0.10	41.5 ± 0.14
Water (µg/L)	0.30 ± 0.02	10.17 ± 0.04	5.20 ± 0.03	4.3 ± 0.10	11.4 ± 0.02
	Site 5				
Sediment (µg/g)	2.33 ± 0.13	8.49 ± 0.04	11.0 ± 0.04	15.0 ± 0.10	15.85 ± 0.10
Water (µg/L)	1.65 ± 0.02	0.6 ± 0.02	4.65 ± 0.02	5.09 ± 0.03	10.3 ± 0.03
	Site 6				
Sediment (µg/g)	32.43 ± 0.02	30.3 ± 0.04	20.0 ± 0.04	27.84 ± 0.04	30.05 ± 0.02
Water (µg/L)	0.3 ± 0.02	0.3 ± 0.02	3.05 ± 0.02	3.85 ± 0.02	4.9 ± 0.03
	Site 7				
Sediment (µg/g)	41.0 ± 0.04	22.9 ± 0.10	23.33 ± 0.02	30.7 ± 0.04	41.5 ± 0.02
Water (µg/L)	0.67 ± 0.01	0.04 ± 0.02	0.35 ± 0.02	3.4 ± 0.03	5.2 ± 0.03

2.4.5.5. Zinc

For Zn, there were significantly higher concentrations in the sediment samples (Table 20) compared to the water samples ($p < 0.05$, $n = 5$) during all five seasons, at all the other sites except at site 3. At site 1, the water concentrations increased gradually from winter 2000 until the following winter. The sediment concentrations decreased from winter to spring, and then increased from summer, through autumn to winter 2001. At site 2, the Zn concentrations in the water samples decreased from winter 2000 to summer, and then increased from autumn to winter 2001. Meanwhile, the sediment concentrations decreased from winter to spring, and then increased from summer, through autumn to winter 2001. At site 5, the Zn concentrations in both the water and sediment samples decreased from winter to summer, then increased again from autumn to winter 2001. The Zn concentrations in the water samples from the reference site (site 6) increased from winter to spring, decreased during summer, then increased again from autumn to winter 2001. The sediment concentrations from site 6 decreased from winter 2000 to spring, and then increased from summer, through autumn to winter 2001. At site 7, both the water and sediment Zn concentrations decreased from winter to spring, then increased again from summer, through autumn to winter 2001.

Table 20: A comparison of Zn concentrations in water (µg/L) and sediments (µg/g) (± SE) from the sites where significant differences were observed (Site 1- Strand; site 2- Gordon's Bay; site 3- Glencairn; site 4- Muizenberg; site 5- Rooiels; site 6- Kleinmond; site 7- Miller's Point)

	Winter 2000	Spring	Summer	Autumn 2001	Winter 2001
	Site 1				
Sediment (µg/g)	38.24 ± 0.02	21.6 ± 0.02	81.25 ± 0.10	85.91 ± 0.01	90.25 ± 0.01
Water (µg/L)	3.35 ± 0.02	4.3 ± 0.03	9.5 ± 0.03	10.12 ± 0.04	12.44 ± 0.02
	Site 2				
Sediment (µg/g)	72.71 ± 0.03	30.77 ± 0.03	56.2 ± 0.10	67.37 ± 0.04	69.44 ± 0.10
Water (µg/L)	16.95 ± 0.02	12.3± 0.03	8.0 ± 0.10	10.95 ± 0.03	14.87 ± 0.02
	Site 4				
Sediment (µg/g)	26.73 ± 0.10	21.54 ± 0.10	100.0 ± 0.03	110.46 ± 0.10	119.55 ± 0.02
Water (µg/L)	30.15 ± 0.03	9.3 ± 0.02	16.65 ± 0.10	17.0 ± 0.04	18.22 ± 0.02
	Site 5				
Sediment (µg/g)	100.0 ± 0.34	90.91 ± 0.29	41.5 ± 0.10	67.67 ± 0.03	80.27 ± 0.04
Water (µg/L)	48.05 ± 0.10	5.6 ± 0.02	5.13 ± 0.02	7.09 ± 0.02	10.5 ± 0.01
	Site 6				
Sediment (µg/g)	59.23 ± 0.02	20.0 ± 0.04	72.73 ± 0.23	77.83 ± 0.10	80.49 ± 0.10
Water (µg/L)	0.3 ± 0.02	0.3 ± 0.02	3.05 ± 0.02	3.85 ± 0.02	4.9 ± 0.03
	Site 7				
Sediment (µg/g)	67.5 ± 0.03	39.36 ± 0.04	46.57 ± 0.10	61.5 ± 0.04	71.75 ± 0.10
Water (µg/L)	4.5 ± 0.02	1.9 ± 0.02	3.55 ± 0.10	6.3 ± 0.03	11.97 ± 0.01

2.4.6. Correlation analyses

The relationships between the water parameters and the heavy metal concentrations in the water and sediments were determined using the Spearman Rank correlation analyses. Generally, there was a significant negative correlation between salinity and the heavy metal concentrations ($p < 0.05$, $r = -0.85$), as well as between pH and heavy metal concentrations.

2.4.7. Contamination factors

The contamination factor for each heavy metal at each site was calculated using the background levels previously determined for False Bay as being: Cd = 0.05; Cu = 3; Ni = 4; Pb =2.5; Zn =17 μg/g (DEAT, 1985). Table 21 shows the contamination factors calculated for the heavy metals from each site, during the different seasons, using the equation used previously by El-Sammak & Aboul-Kassim (1999):

CF = *Metal concentration in sediments/ background levels for shallow marine sediments*

Generally, the values decreased from winter to spring, then increased again from summer to winter 2001. At most sites, the contamination values for Cd were higher, followed by those of Pb and Ni.

2.4.8. Pollution load indices (PLI)

The pollution load indices calculated for the sediments of the different sites during different seasons are shown in Table 22. The pollution loads tended to decrease during spring, and increase again during summer. Sites 2 and 4 had the highest pollution loads, while site 3 had the lowest during all seasons.

TABLE 21 Sediment contamination factors (CF) calculated for the various heavy metals at different sites during five seasons, based on DEAT (1985) background levels

Sites	Cd	Cu	Ni	Pb	Zn
Site 1					
Winter '00	147.2	5.03	3.05	6.06	2.25
Spring	30.8	0	4.38	0.8	1.27
Summer	60	0	5.63	4.03	4.78
Autumn	61.8	0.41	7.7	6.72	5.05
Winter '01	103.2	2.26	8.25	6.8	5.31
Site 2					
Winter '00	60.6	1.06	6.06	24.3	4.28
Spring	20.2	0	4.23	15.38	1.81
Summer	105	0	7.56	14.03	3.31
Autumn	123.4	0.33	9.32	14.42	3.96
Winter '01	138	2.32	9.5	16.2	4.09
Site 3					
Winter '00	4.2	0.39	1.61	4.34	0.83
Spring	2	0	0.13	1.47	2.35
Summer	0	0	3.26	12.03	3.82
Autumn	4.2	2.02	4.18	13.86	1.23
Winter '01	24.2	20.3	4.98	13.9	2.05
Site 4					
Winter '00	247.2	1.76	4.55	11.36	1.57
Spring	55.4	0.47	2.35	18.91	1.27
Summer	215	0	12.5	11.46	5.88
Autumn	209.2	2.49	7.72	15.09	6.5
Winter 01	235.8	2.82	11.71	16.6	7.03
Site 5					
Winter 00	140	0	9.29	2.93	5.88
Spring	6.4	1.57	0.8	3.4	5.35
Summer	2	0	2.5	4.4	2.44
Autumn	25	1.17	3.58	6	3.98
Winter 01	67.8	1.39	8.58	6.34	4.72
Site 6					
Winter 00	56	0.72	7.31	12.97	3.48
Spring	12.2	0.1	3.15	12.12	1.18
Summer	20	0	2.19	8	4.28
Autumn	35	0.74	3.45	11.14	4.58
Winter 01	42	1.26	5.99	12.02	4.74
Site 7					
Winter 00	4	3.67	7.38	16.4	3.97
Spring	6	0.9	3.95	9.16	2.32
Summer	26	0.65	2.41	9.33	2.74
Autumn	31.8	3.83	2.9	12.28	3.62
Winter 01	33.4	4	7.88	16.6	4.22

Table 22: Pollution load indices for the five heavy metals at the different sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	4.98	2.68	5.79	5.81	9.3
Site 2	8.35	4.73	8.19	7.37	11.5
Site 3	1.57	0.98	2.72	3.6	5.87
Site 4	8.12	4.3	11.26	13.16	15.55
Site 5	7.41	2.71	2.22	4.78	7.53
Site 6	6.68	2.23	4.32	5.39	7.1
Site 7	5.88	3.4	4.01	6.91	9.41

2.4.9. Comparison of present and previous heavy metal concentrations

Due to the lack of data on historic levels of heavy metals, the water concentrations of the present study were compared to those measured at a few stormwater outlets during 1988 (Table 23). Generally, the ranges of the heavy metal concentrations of the present study were higher than the previous ranges for Cd and Ni, while they were lower for the other metals.

Table 23: Comparison of present water concentrations (µg/L) to previous levels measured during 1988 (Taljaard et al., 2000)

Metal	Present ranges (µg/g)	Previous ranges µg/g
Cd	ND- 10.40	0.01 - 0.2
Cu	ND- 4.65	1.3 - 18.3
Ni	ND · 10.74	1.0 - 7.9
Pb	0.15- 14.00	0.7 - 20.1
Zn	3.35 - 20.30	4.3 - 81.0

2.5. DISCUSSION

In the present study, higher concentrations of heavy metals occurred during winter than at other times of the year (Tables 2, 3, 5, 11 and 12). This might have been caused by the increased amount of runoff during the winter rainfall in the area (Table 10), which may, in turn, have resulted in the increased mobilization of heavy metals from diffuse sources (Neal et al., 2000). According to Forstner & Wittman (1979), strong fluctuations in heavy metal concentrations in the sediments can be due to storm water drainage, varying sedimentation rates and unequal distribution of metals. As the seasons progressed during the present study, local dilution effects may have taken over, resulting in the decreased concentrations of heavy metals during spring or summer. The higher pollution load indices obtained during winter (Table 22) support these findings. Since temperature also influences metal concentrations in the ambient environment (Abbe et al., 2000), this may also explain the variations in the metal concentrations during the different seasons, and the observed negative correlation between temperature and water concentrations of Cd, Cu and Ni.

According to Kinne (1984), municipal wastewater often contains heavy metals from industrial discharge, thus becoming a source of Cd contamination when discharged into coastal waters. In the present study, the presence of Cd (Tables 2 and 11) may indicate a strong input of industrial discharge, especially at sites 1 and 2, where a chemical factory, SOMCHEM, is situated. The effluent from the factory was previously discharged intermittently into False Bay near site 1 until 2000 (Cookson, pers. comm., 2003), and this may account for the elevated Cd concentration at these sites.

Treated effluent from the Gordon's Bay Wastewater Treatment Works is discharged directly into the Sir Lowry's Pass River which eventually drains into False Bay near site 2 (Taljaard et al., 2000), and may also contribute to the Ni, Pb and Zn concentrations at this site. Cd is closely related to Zn, and will be found wherever Zn occurs in nature (Friberg et al., 1974). Cd and Zn contamination have been observed

in most industrial areas, with major sources being metallurgic and chemical factories, the fisheries industry, agricultural fertilizers and domestic waste landfills (Barcellos & Lacerda, 1994).

According to Barnes (1977), sewage outfalls of large urban-industrial areas carry a wide range of chemical effluents and runoff, and may discharge directly onto the beach, or terminate some distance below the low-water mark. In False Bay, fourteen sewage works exist (Anon., 1983), as well as a number of stormwater outlets which discharge directly into the bay at all the sites (Taljaard et al., 2000), and these sources may also contribute to the heavy metal contamination in the bay. Thus, the presence of Cu (Tables 3 and 12) may indicate anthropogenic influences related to the input of untreated domestic and agricultural sewage (Kinne, 1984), as well as industrial discharge (Molisani et al., 1999). The widespread use of Cu as a constituent of herbicides and fungicides in the Lourens River and Sir Lowry's Pass catchments which drain at sites 1 and 2 (Heinecken et al., 1983) may account for the Cu contamination at these sites. According to Heinecken et al. (1983), the sewage pumping stations near Sandvlei River (Figure 1) previously discharged industrial effluent from a sawmill, a textile mill, electronics and engineering factories. These may have contributed to the elevated heavy metal concentrations and pollution loads at site 4, into which the Sandvlei River discharges (Taljaard et al., 2000).

Landfill leachates are often contaminated with organic acids (Taljaard et al., 2000), and can be a source of Cu contamination when these acids mix with Cu. The presence of solid waste dumpsites at sites 2 and 7 may account for the elevated concentrations of Cu at these sites (Tables 3 and 11). Microbial contamination is a common occurrence at False Bay during the rainy season (Taljaard et al., 2000), and is caused by the discharge of raw sewage from the informal settlements in the northern shores of the bay, or pump breakdowns. This leads to high levels of organic matter, as seen in the presence of green algae covering the rocky shore, especially at site 1.

According to Rainbow (1995), heavy metals tend to accumulate in organically rich sediments. This may explain the elevated concentrations of Cu and Ni in the water and sediment samples from sites 1, 4 and 5 (Tables 3, 4, 12 and 13), since these heavy metals have a strong affinity for organic matter (Taljaard et al., 2000). Cu is often used as a wood preservative to reduce damage to marine structures by boring organisms (Adler-Ivanbrook & Breslin, 1999). The leaching of Cu from copper-treated wooden piers and anti-fouling paints used on boats may be another source of Cu contamination at sites 2 and 7, which have a fishing harbour and yachting club respectively (Taljaard et al., 2000). A railway line which runs very close to the shore between sites 3 and 4 (Taljaard et al., 2000) may also contribute to Cu contamination by leaching of copper-based paints that are often used in preserving railway trestles, as found by Goh & Chou (1997) and Botton et al. (1998) elsewhere.

The presence of Pb in the water and sediment samples (Tables 5 and 14) may be the result of road runoff containing Pb from motor vehicle exhaust emissions, which is discharged into False Bay via the stormwater outlets (Taljaard et al., 2000). Another possible source of Pb may be the boat and yacht fuel, as well as the lead sinkers which are used by anglers in their recreational activities (Taljaard et al., 2000). According to Charlesworth et al. (1999), anthropogenic Pb is mostly associated with the slowest-settling fraction of particulate matter, and so has the potential to be transported far from its source until it reaches areas of high water column stability or areas of weak currents. The sheltered nature of site 2 (Figure 1) makes it vulnerable to pollution as the current-borne pollutants are eventually deposited in this enclosed, low-energy area (Taljaard et al., 2000). This may be exacerbated by the convergence of the clock-wise southeasterly and the anti-clockwise north-westerly winds which blow during summer and winter respectively (Taljaard et al 2000) (Figure 2). According to Jury (1991), the seasonal change from southeasterly to north-westerly winds is an important feature of circulation in the study area, and this contributes to the sharp gradients in wind speed and temperature, which may, in turn, influence heavy metal distribution.

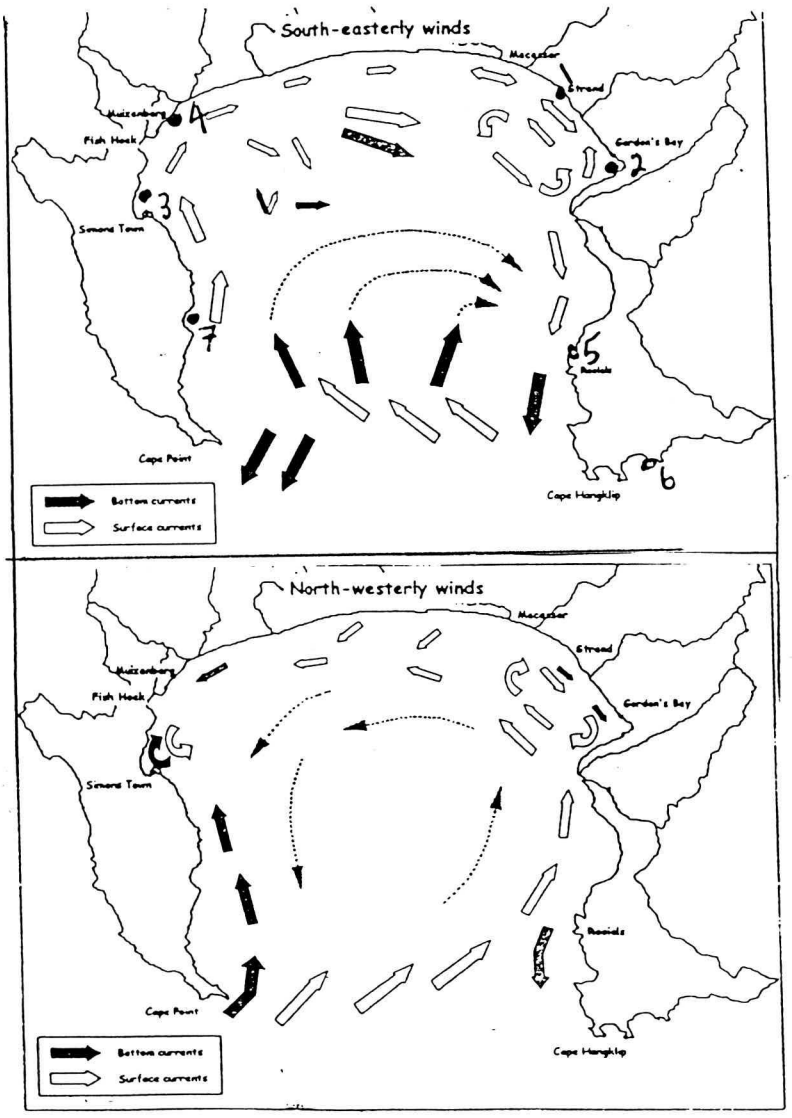

Figure 2: Diagram showing the surface and deep water flows under the two wind regimes in False Bay (Taljaard et al., 2000)

Long-distance transport of airborne Pb from the heavily populated and industrial areas of the Cape Metropolitan area to the False Bay shoreline by the strong southeasterly and the north-westerly wind regimes may also act as a source of Pb in the study area (Taljaard et al., 2000). This may account for the higher Pb concentrations at sites 3, 4

and 7 during winter (Table 14), as these sites are situated along the western margin of the bay.

A study carried out to assess the hydrodynamics in False Bay (Taljaard et al., 2000) demonstrated a tendency for a closed circulation taking place at site 2, which becomes cyclonic under the north-westerly winds (Figure 2). Because of the sheltered nature of this site (Figure 1), there is evidence of accumulation of sediments and contaminants in this area (Taljaard et al., 2000), which may have contributed to the high levels of Pb measured in the sediments from this site during winter 2000 (Tables 5 & 14).

Darracott & Watling (1975) found that pollutant concentrations in the water fluctuated widely under conditions of variable rainfall. According to Morant (1991), all the catchments which drain into False Bay are small, with the result that river flow becomes very sensitive to rainfall. The higher amount of rainfall measured in False Bay during winter 2001 (Table 10) may therefore account for the higher pollution loads obtained during this season at most sites (Table 22) as well as for the significant seasonal variations observed in the heavy metal concentrations in the present study.

According to Shriadah (1998), Ni and Zn concentrations increase in areas which receive large quantities of municipal and industrial wastewater, and from fishermen, with Ni being also associated with the boat repair industry (DEAT, 1985), and which may account for the presence of Ni in the fishing areas such as sites 2, 4, 5, 6 and 7 (Tables 4, 6 and 13). A probable source of Zn at sites 2 and 7 may be the boating activities such as sandblasting of boats, effluent discharge from fishing activities, and bilge waters (Ruelas-Inzunza & Paez-Osuna, 1998).

According to Deacon & Driver (1999), heavy metals are unevenly distributed in the aquatic environment, which in the present study was also observed in the significantly higher heavy metal concentrations in the sediments than those of the water

concentrations (Tables 16- 20). The results of the present study were also in agreement with the views of others (Sarki et al., 1995; Slotton & Reuter, 1995; Deacon & Driver, 1999) who found that heavy metal contamination in aquatic systems were characterized by higher levels in the sediments than in the overlying waters.

Seawater is not neutral but slightly alkaline, with a pH ranging between 8.00 and 8.5 (Kinne, 1984) and which is largely determined by the bicarbonate or borate concentrations (Parsons & Takahashi, 1973). In the present study, the decrease in the water pH during winter (Table 8) may have been caused by various atmospheric contaminants present in acidic precipitation and runoff (Philp, 1999). No previous monitoring program or research into the effect of rainfall on pH in False Bay could be found to verify the results of the present study. The increased precipitation and the resultant high runoff rate may, according to O'Loughlin et al. (2000) also contribute to the reduction in pH if accompanied by the presence of dissolved humic acids in soils. Previous studies (Bermejo et al., 2002; Otero & Macias, 2002) have shown that changes in the tidal cycle in the intertidal zone significantly affects the pH and the processes of synthesis and oxidation of metal sulfides, which may, in turn, affect the solubility and bioavailability of metals.

The salinity of seawater is about $35^{o}/_{oo}$ (Kinne, 1984), although differences in surface salinities are sometimes caused by variations in the extent of evaporation, quantity of rainfall and inflow of fresh water from river. In the present study, the lower salinities recorded at sites 1, 5 and 6 (Table 9) may be an indication of strong fresh water inflow from the Lourens, Rooiels and Kleinmond Rivers respectively. According to Hops (1990), decreased salinity leads to an increase in metal mobility, which may explain the higher concentrations of heavy metals in sediments and water samples during winter, compared to the other seasons.

During the early 1980s, there was relatively little development and restricted access to site 5 (Heinecken et al., 1983). Studies which were carried out previously on the pollution input by the various catchments into False Bay indicated that the Rooiels catchment contributed little to the contamination load of the bay. However, during the late 1980s, a weapons testing site was established in the catchment area of site 5 by SOMCHEM for the testing of cannon propellants and rockets for the defence force of the previous government (Cock & McKenzie, 1998). According to Migliorini et al. (2003), shooting ranges used for military training and sport can constitute important sources of heavy metal contamination, due to the oxidation and deterioration of pellets which make metallic Pb and other metals become bioavailable. In the present study, therefore, acidic fallout and unexploded mortars and shells, if used in this area, may have contributed to the accumulation of heavy metals in the area, although no evidence was found to confirm this. Recently, there has been large-scale construction of holiday homes at site 5, resulting in an upsurge in the number of residences in the area. Construction activities such as welding, cutting, brazing and blasting may contribute to the heavy metal contamination in the area (Tables 2, 4, 8, 11-15). The use of septic tanks for sewage disposal at this site may also be responsible for the leaching of anti-corrosive substances in these tanks into groundwater, and eventually into the sediments.

The contamination factor for Cd in the sediments was generally higher than the other metals at all the sites, followed by that for Pb and Ni (Table 21), which may suggest a strong input of industrial discharge containing more Cd than the other heavy metals, as suggested by El-Sammak & Aboul-Kassim (1999) in their observations elsewhere. The heavy metal concentrations in the present study were compared to the allowable limits given in the South African Water Quality Standards (DWAF, 1995), and it was found that Cd and Zn occasionally exceeded the recommended limits of 4 and 25 μg/g respectively, especially at sites 3 and 5 (Tables 11 and 15). In the absence of guidelines for marine sediments in South Africa (Taljaard et al., 2000), the results of the present study were compared to the threshold-effect levels (TEL) and the probable-effect levels (PEL) given elsewhere (Kinne, 1984; Deacon & Driver, 1999;

Carr et al., 2001). The TEL represent the concentrations below which adverse effect to aquatic biota are not expected to occur, while the PEL is the level above which adverse effects to aquatic biota are predicted to occur. In the present study, the TEL and PEL for Cd (0.596 and 3.53 µg/g respectively) were both exceeded (Table 2). The TEL for Pb (35 µg/g) was exceeded at sites 2, 3, 4, 6 and 7 (Table 5) while the PEL (91.3 µg/g) was not exceeded. Although the reductions observed in the ranges of Cu and Zn levels measured in the present study compared to those of previous surveys in False Bay (Table 23) cannot be explained and may be the results of analytical differences, the slight reduction in the range of Pb values may be related to the introduction of unleaded petrol in South Africa in 1998.

As a result of the partially enclosed nature of False Bay (Taljaard et al., 2000), there may be poor circulation in the bay, and this, combined with the residence time of between four and six days (Taljaard et al., 2000) may contribute to the higher pollution levels of the sites which are situated within the bay, compared to the reference site (site 6) (Table 22). Although site 6 is a fishing village, the contamination impacts associated with the fishing activities which take place at this site may have been reduced by the rapid dissipation of pollutants by currents in the open sea.

2.6.CONCLUSION

Heavy metal concentrations in the sediments were higher than in the overlying waters. Contamination appeared to be associated with the northern shore between sites 1 and 4, where the more populated and industrialized catchments in the bay occur. Although the heavy metal concentrations in the present study were not extreme compared to the concentrations measured in other parts of the world, the concentrations of Cd, Pb and Ni were occasionally higher than the recommended or permissible levels. The water pH and water salinity appear to be important factors in the seasonal variation of heavy metals, since a reduction in both caused increased solubility and mobility of heavy metals. Pollution in the study area seems to be influenced by the various sources, with domestic wastewaters probably constituting the largest source of elevated heavy

metals. Seasonal and spatial distribution were, generally, specific for each heavy metal. It may be concluded that there has been an increase in the levels of Cd and Ni concentrations since the previous water quality surveys.

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CHAPTER 3 – HEAVY METAL CONCENTRATIONS IN THE BARNACLE *TETRACLITA SERRATA* (CRUSTACEA: CIRRIPEDIA) FROM FALSE BAY

3.1. INTRODUCTION

As heavy metals are increasingly being introduced into the environment, their uptake by invertebrates living in contaminated habitats is an important pathway by which heavy metals enter the animal food chain (Pilgrim & Hughes, 1994). Aquatic organisms living in close contact with sediments where metals accumulate are known to concentrate a whole range of contaminants in their soft tissues (Giamberini & Pihan, 1997), thus can be used as sentinel organisms in pollution monitoring. The major routes of input of contamination depend on the dietary lifestyle of each species, such as via the suspended particulate matter or water column for filter feeders such as barnacles (Lynch & Wiseman, 1998).

The term "biomonitor" refers to a species that accumulates pollutants in its tissues, and may be analyzed as a measure of bioavailability of metals in the surroundings (Rainbow, 1995). Heavy metals are accumulated by marine organisms to very high tissue concentrations, with individual biomonitors responding differently to the different sources of bioavailable metals, and different species from the same habitat showing different patterns of metal accumulation according to the different uptake routes that exist (Rainbow, 1995). The mussel *Perna viridis* and the barnacle *Balanus amphitrite* collected from Hong Kong waters, for example, were found to accumulate up to 153 and 11 990 µg/g Zn, respectively (Rainbow, 1995), while the barnacle *Tetraclita squamosa* accumulated up to 6963 µg/g Zn (Rainbow, 2002). Thus, biomonitors allow for comparisons to be made over space and time, since they provide an integrated measure of the ecotoxicologically significant fraction of heavy metals in the surrounding water (Rainbow et al., 2000).

According to Boening (1999), the accumulation of heavy metals within each individual organism can be a function of age, sex, size, feeding activity and reproductive state. Thus, controls should be included where biomarkers are used. Where fixed surveys are done, the inclusion of a pristine sample site is important.

Where animals for biomarker work are collected randomly and these factors are not known, interpretation of results should be made with the possible variation as a result of this kept in mind.

Different regulatory and sequestration mechanisms occur among marine invertebrates (Phillips & Rainbow, 1988). Some are strong regulators, able to maintain tissue concentration to a constant level within specific ranges which are close to metabolic requirements over a range of ambient concentrations (Phillips & Rainbow, 1988). Partial regulators are able to accumulate in direct proportion to ambient concentrations, with storage occurring in the kidney and hepatopancreas (Phillips & Rainbow, 1988). Others are unable to match the uptake and excretion rates, thus becoming net accumulators.

A stationary organism is continuously exposed to its immediate environment, thus the concentrations of heavy metal within its tissues reflect the average concentrations of those elements in the ambient environment over a longer period (Schulz-Baldes, 1974). Through biological amplification, some species may build up concentrations of metals which are present at low amounts in the environment to levels that are harmful to both organisms and human consumers, and which exceed public health standards (Marcovercchio, 1993).

The suitability of benthic organisms as biomonitors of pollution stem from characteristics such as their sedentary lifestyle, long life spans that integrate contamination over time, and their differential levels of tolerance (Kiffney & Clements, 1993). Among crustaceans, barnacles are the most able to fulfill the role of biomonitors as they are the most sedentary and long-lived, often attaining a mean age of 3 years in the lower vertical range, and 8 years in the upper limit of their distribution in the vertical zone (Griffiths & Branch, 1991). They are strong net accumulators of trace metals (Rainbow, 1995), and are found in different locations with different salinities (Blackmore, 1999) and where contrasting activities exist (Ruelas-Inzunza & Paez-Osuna, 1998).

Barnacles are able to add newly absorbed metals to existing permanent body stores, and exhibit uptake-storage mechanisms of metal detoxification that are unlike other marine crustaceans (Linthurst et al., 2001). According to Blackmore (1999), barnacles are microphagous feeders which ingest metal-rich particles with the large volumes of water passing across the permeable surfaces of their cirri. Since they lack a hepatopancreas, which is the storage organ for heavy metals in several other organisms (Masala et al., 2002), barnacles accumulate metals into granular deposits containing calcium pyrophosphate.

The False Bay coastline is not homogenous, being characterised by sweeping sandy beaches on the northern shoreline, while to the south the bay opens to the continental shelf and is flanked by rugged rocky shores (Taljaard et al., 2000). The varied temperature regime in the study area has resulted in a remarkably diverse distribution of the biota, with the warmer conditions at the northern end favouring south coast species, whereas the cooler conditions at the southern end allow the growth of cool-temperate-tolerant west coast species (Taljaard et al, 2000). As a result of these factors, the animal species under study were found not to have a uniform distribution at all the study sites.

Several authors have shown barnacles to be strong net accumulators of copper and zinc (Walker, 1977; Ruelas-Inzunza & Paez-Osuna, 1998), and cadmium (Beyer et al., 1996). The aim of this part of the study was to determine the heavy metal concentrations in the barnacles obtained from different sampling points along the False Bay coastline, in order to determine their reliability as biomonitors of heavy metal contamination by comparing body loads and environmental concentrations.

3.2. MATERIALS AND METHODS

3.2.1. Animal sampling

Whole-animal samples of *Tetraclita serrata* were collected seasonally from the seven sampling sites where the species occurs abundantly along the False Bay coastline (Figure 1, Chapter 2). The animals were collected at low spring tides from the rocky substrate in the intertidal zone. Between 50 and 60 individuals were collected per sample in order to provide sufficient dry tissue for five replicate analyses. The barnacle specimens were dislodged from the rocky substrate with a stainless steel knife. To prevent loss of body fluids during transportation to the laboratory, the animals were placed upside down in plastic buckets containing site water (Ireland, 1974). The water temperature, pH and salinity were measured using a thermometer, pH meter (Crison micro pH 2001) and salinometer respectively. The animal samples were killed by freezing at -20^{0} C, and stored until further analysis.

3.2.2. Heavy metal analysis

Each whole-body (soft tissues and shell) composite sample of 50-60 pooled individuals was weighed and oven-dried to a constant weight at 60^{0} C for 48 hours. Since the main objective of this study was to gain an insight into the broad picture of site-specific variations in metal concentrations, and the potential for biomonitoring of each species as a group, and not individually-based variations *per se*, the individual body samples were pooled for the purposes of this study. It is common practice to pool organisms into composite samples before analysis (Lobel et al., 1982), with the benefits being the ability to process a large number of organisms in a given time, resulting in cost-effective metal analysis as well as to reduce size-related metal variations (Powell & White, 1990). Pooling of samples is also recommended to reduce the effects of small-scale and temporal variance (Birch et al., 2001). The whole-body samples were homogenized by grinding to a fine powder with pestle and mortar. Triplicate tissue aliquots of 0.2- 0.5 g were placed in acid-cleaned test tubes and digested with 10 ml of nitric acid (55%), and then left to stand overnight. The same procedure for acid digestion was followed as described in chapter 2 (p. 18) for the sediment and water samples.

Blank determinations were run concurrently. Heavy metal concentrations (Cd, Cu, Ni, Pb and Zn) were determined using the flame AAS method (Szefer et al., 1997).

3.2.3 Statistical analysis

One-way ANOVA was used to determine the seasonal and spatial variations in the heavy metal concentrations of the body samples. The body concentrations were compared to the water concentrations measured during the same period at the different sites (Chapter 2) using t-test. Pearson correlation analyses were used to determine the relationship between the water parameters and whole-body heavy metal concentrations, as well as between the water and body concentrations.

3.3. RESULTS

3.3.1. Heavy metal in the barnacle body samples

3.3.1.1. Cadmium

The mean body Cd concentrations measured during winter 2000 varied between 0.78 (± 0.10) and 70.67 (± 0.10) µg/g dry weight, with the highest concentrations being measured in the samples from site 5 (Table 24). At most sites, the mean concentrations decreased slightly during spring and summer. During autumn, the mean body concentrations of Cd increased slightly at most sites, except at sites 5 and 7, where the concentrations decreased in the former, and where no Cd was detected in the latter. During winter 2001, the mean concentrations increased slightly at all the sites, ranging between 1.05 (± 0.02) and 25.95 (± 0.04) µg/g dry weight. One-way ANOVA showed that there were significant spatial and seasonal differences ($p < 0.05$, $n = 5$ pool of 50 samples).

Table 24: Mean Cd concentrations (µg/g ± SE, n = 5) in body samples of barnacles from different sites obtained during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	4.39 ± 0.02	1.39 ± 0.02	1.11 ± 0.03	3.60 ± 0.04	4.80 ± 0.02
Site 2	3.83± 0.03	1.35 ± 0.04	1.25 ± 0.02	1.75 ± 0.10	2.01± 0.03
Site 3	0.78 ± 0.10	1.50 ± 0.02	0.89 ± 0.10	4.12 ± 0.03	4.92 ± 0.10
Site 4	5.39± 0.04	1.0 ± 0.03	0.75 ± 0.03	8.27 ± 0.10	9.01 ± 0.04
Site 5	70.67 ± 0.10	68.89± 0.10	42.22± 0.11	20.67± 0.03	25.95 ± 0.04
Site 6	10.0 ± 0.03	5.97 ± 0.10	0.97 ± 0.02	6.60 ± 0.03	10.75 ± 0.02
Site 7	18.0 ± 0.03	2.80 ± 0.04	2.0 ± 0.04	ND	1.05 ± 0.02

3.3.1.2. Copper

There were significant seasonal variations in the mean Cu concentrations of the barnacle body ($p < 0.05$), with the means ranging between undetectable levels and 40.25 (± 0.01) µg/g. The values decreased from winter 2000 to spring, and were below detectable levels at sites 1, 2 and 4 (Table 25). At site 6, the mean body concentrations were significantly higher than those of the other sites during winter 2000 and spring. During summer, Cu was not detected in the barnacles from sites 1, 4 and 6. The mean body concentrations tended to increase from autumn to winter 2001 at all sites.

Table 25: Mean Cu concentrations (µg/g ± SE, n = 5) in body samples of barnacles from different sites obtained during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	ND	ND	ND	3.28 ± 0.03	8.75 ± 0.04
Site 2	1.70± 0.04	ND	0.42 ± 0.03	1.29 ± 0.03	7.50± 0.03
Site 3	3.28 ± 0.03	1.0 ± 0.02	1.17 ± 0.04	4.12 ± 0.03	4.51 ± 0.03
Site 4	3.85± 0.03	ND	ND	10.38± 0.10	10.47± 0.03
Site 5	4.0 ± 0.04	3.75± 0.04	0.93 ± 0.04	3.50± 0.04	11.0 ± 0.10
Site 6	40.25± 0.03	32.64± 0.02	ND	5.50 ± 0.04	11.50 ± 0.10
Site 7	7.0 ± 0.10	1.57 ± 0.10	1.25± 0.04	ND	7.50 ± 0.04

3.3.1.3. Nickel

The mean Ni concentrations in the barnacles ranged between undetectable levels and 17.31 (± 0.04) µg/g. During winter 2000, Ni was detected in the specimens from only three sites (Table 26). During spring, the mean concentrations increased significantly ($p < 0.05$) in the barnacles from sites 3, 4, 5 and 6 while remaining below detection levels in those from sites 1, 2 and 7. During summer, the mean body concentrations were below detection limits at sites 1, 2 and 4 while they increased at the other sites. During autumn, the mean concentrations increased progressively to significantly higher concentrations in winter 2001.

Table 26: Mean Ni concentrations (µg/g ± SE, n = 5) in body samples of barnacles from different sites obtained during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	1.10 ± 0.04	ND	ND	1.25 ± 0.02	5.16 ± 0.04
Site 2	2.40± 0.04	ND	ND	1.08 ± 0.04	1.90± 0.10
Site 3	0.77 ± 0.04	1.0 ± 0.04	5.60 ± 0.10	8.30 ± 0.03	15.22 ± 0.03
Site 4	ND	0.86 ± 0.10	ND	17.31± 0.03	9.42± 0.02
Site 5	ND	1.69± 0.04	6.11 ± 0.03	10.17 ±0.10	12.50± 0.04
Site 6	ND	1.67± 0.04	9.52 ± 0.04	13.0 ± 0.10	15.50 ± 0.03
Site 7	ND	ND	1.80± 0.04	2.75 ± 0.04	5.50 ± 0.10

3.3.1.4. Lead

The mean Pb concentrations (Table 27) measured in the barnacles during winter 2000 varied between 0.77 µg/g (± 0.04) at site 4 and 9.00 µg/g (± 0.04) at site 7. During spring, the mean concentrations ranged between undetectable levels and 1.11 (± 0.01) µg/g. The mean concentrations measured during summer ranged between undetectable levels and 12.60 (± 0.04) µg/g, with the latter being measured in the samples from site 7. During autumn, the mean concentrations ranged between 0.57 (± 0.10) and 18.75 (± 0.03) µg/g, with the highest being measured in the samples from site 7. The mean concentrations obtained during winter 2001 varied between 2.80 (± 0.04) and 20.00 (± 0.10) µg/g, with the highest concentrations being measured at sites 5 and 7. One-way ANOVA showed that the mean concentrations of Pb obtained in the body samples during winter 2001 were significantly higher ($p < 0.001$, n = 5 pool of 50 samples) than those measured during other seasons. There were also significant spatial differences in the mean Ni concentrations of the body samples ($p < 0.001$).

Table **27:** Mean Pb concentrations (µg/g ± SE, n = 5) in body samples of barnacles from different sites obtained during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	3.13 ± 0.03	0.67 ± 0.04	ND	16.41 ± 0.04	20.47 ± 0.10
Site 2	5.10± 0.10	ND	ND	0.57 ± 0.10	2.80± 0.04
Site 3	1.96 ± 0.04	ND	ND	12.36± 0.10	15.33 ± 0.04
Site 4	0.77 ± 0.04	ND	ND	9.62± 0.10	11.54 ±0.03
Site 5	3.33 ± 0.10	1.11± 0.01	0.56 ± 0.03	17.33 ±0.03	20.0± 0.10
Site 6	6.0 ± 0.04	ND	ND	1.50 ± 0.04	3.750 ± 0.03
Site 7	9.0 ± 0.04	ND	12.60 ±0.04	18.75 ± 0.03	20.0 ± 0.10

*ND- not detected

3.3.1.5. Zinc

The mean body concentrations of Zn (Table 28) ranged between 27.55 (± 0.10) and 209.00 (± 0.10) µg/g, and tended to decrease during spring and summer, while they increased progressively through autumn to winter 2001. The mean body concentrations in the barnacles obtained from site 6 were significantly higher ($p < 0.05$) than those from other sites during winter 2000, spring and autumn. The mean concentration of samples from site 5 was significantly higher than those of the other samples during summer ($p < 0.001$), while the mean concentration of site 4 samples was significantly higher than those from the other sites during winter 2001 ($p < 0.001$). One-way ANOVA showed that there were significant seasonal variations ($p < 0.05$, n = 5 pool of 50 samples) between the Zn mean concentrations of the barnacle body samples.

Table 28: Mean Zn concentrations (µg/g ± SE, n = 5) in body samples of barnacles from different sites obtained during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 1	57.97± 0.10	30.33± 0.03	43.75 ± 0.03	46.88 ± 0.10	65.63 ± 0.02
Site 2	93.26 ± 0.03	38.85 ± 0.03	57.71 ± 0.02	65.24± 0.03	87.0± 0.04
Site 3	69.22± 0.03	37.0 ± 0.10	27.55 ± 0.10	104.90 ± 0.03	127.45 ± 0.02
Site 4	87.69± 0.03	64.29 ± 0.04	52.31 ± 0.10	144.81 ± 0.04	179.0 ±0.10
Site 5	66.94± 0.03	50. 0 ± 0.10	62.22 ± 0.10	62.50 ±0.03	68.90 ± 0.03
Site 6	209.0 ± 0.10	145.28 ± 0.03	40.81 ± 0.10	160. 0 ± 0.10	170.55 ± 0.10
Site 7	160.05 ± 0.10	140.32 ± 0.03	36.60 ±0.10	120.75± 0.10	138.25 ± 0.10

3.3.2. Comparison of the water and body concentrations

3.3.2.1. Cadmium

The mean Cd body concentrations of barnacles were significantly higher ($p < 0.05$) than those of the water samples during most of the seasons sampled (Table 29). During winter 2000, the mean body concentration at site 5 was nearly 10 times as high as the water concentration, while at site 6 it was 53 times as high as the water concentration. During spring, the mean body concentration at site 5 was as high as 360 times that of the water concentration. The Pearson correlation analysis showed that there was significant positive correlation between the Cd concentrations in water and body samples ($p < 0.001$, $r = 0.97$).

3.3.2.2. Copper

For Cu, the mean body concentrations of the barnacles were significantly higher ($p < 0.001$) than the water concentrations (Table 30) during winter 2001. At site 2, the mean body concentration was 7 times higher than the water concentration. At site 5, the body concentration was 6 times higher than the water concentrations, while at site 6 the body concentrations were 5 times higher than the water concentrations. A significant negative correlation was found between the water pH and the body Cu concentrations ($p < 0.05$, $r = -0.91$).

Table 29: A comparison of Cd concentrations in water (µg/L) and barnacle body samples (µg/g ± SE, n = 5) (Site 1- Strand; site 2- Gordon's Bay; site 3- Glencairn; site 4- Muizenberg; site 5- Rooiels; site 6- Kleinmond; site 7- Miller's Point)

Site 1

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Body	4.39 ± 0.02	1.39 ± 0.02	1.11 ± 0.03	3.60 ± 0.04	4.80 ± 0.02
Water	0.15 ± 0.02	0.20 ± 0.02	1.05 ± 0.03	2.0 ± 0.10	2.49 ± 0.10

Site 2

Body	3.83 ± 0.03	1.35 ± 0.04	1.25 ± 0.02	1.75 ± 0.10	2.01 ± 0.03
Water	0.65 ± 0.03	0.04 ± 0.01	1.0 ± 0.02	1.94 ± 0.04	2.47 ± 0.10

Site 3

Body	0.78 ± 0.10	1.50 ± 0.02	0.89 ± 0.10	4.12 ± 0.03	4.92 ± 0.10
Water	0.05 ± 0 01	0.15 ± 0.02	3.24 ± 0.10	6.60 ± 0.10	6.94 ± 0.04

Site 4

Body	5.39 ± 0.04	1.0 ± 0.03	0.75 ± 0.03	8.27 ± 0.10	9.01 ± 0.04
Water	0.75 ± 0.04	0.55 ± 0.03	1.37 ± 0.10	2.01 ± 0.02	2.40 ± 0.02

Site 5

Body	70.67 ± 0.10	68.89 ± 0.10	42.22 ± 0.11	20.67 ± 0.03	25.95 ± 0.04
Water	10.40 ± 0.10	0.19 ± 0.02	0.13 ± 0.02	0.97 ± 0.04	1.77 ± 0.04

Site 6

Body	10.0 ± 0.03	5.97 ± 0.10	0.97 ± 0.02	6.60 ± 0.03	10.75 ± 0.02
Water	0.19 ± 0.03	0.40 ± 0.03	0.60 ± 0.02	1.20 ± 0.03	2.09 ± 0.02

Site 7

Body	18.0 ± 0.03	2.80 ± 0.04	2.0 ± 0.04	ND	1.05 ± 0.02
Water	ND	ND	0 15 ± 0.02	1.49 ± 0.02	1.88 ± 0.02

*ND- not detected

Table 30: A comparison of Cu concentrations in water (µg/L) and barnacle body samples (µg/g ± SE, n = 5) (Site 1- Strand; site 2- Gordon's Bay; site 3- Glencairn; site 4- Muizenberg; site 5- Rooiels; site 6- Kleinmond; site 7- Miller's Point)

Site 1

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Body	ND	ND	ND	3.28 ± 0.03	8.75 ± 0.04
Water	ND	ND	0.67 ± 0.02	1.10± 0.03	2.09 ± 0.10

Site 2

Body	1.70 ± 0.04	ND	0.42 ± 0.03	1.29 ± 0.03	7.50 ± 0.03
Water	1.55 ± 0.02	ND	0.67 ± 0.02	0.95 ± 0.04	1.15 ± 0.02

Site 3

Body	3.28 ± 0.03	1.0 ± 0.02	1.17 ± 0.04	4.12 ± 0.03	4.51 ± 0.03
Water	2.20 ± 0.02	ND	0.12 ± 0 02	1.84 ± 0.03	2.30 ± 0.03

Site 4

Body	3.85 ± 0.03	ND	ND	10.38± 0.10	10.47± 0.03
Water	0.60 ± 0.04	0.60 ± 0.02	0.40 ± 0.03	1.22 ± 0.01	3.10 ± 0.02

Site 5

Body	4.0 ± 0.04	3.75 ± 0.04	0.93 ± 0.04	3.50 ± 0.04	11.0 ± 0.10
Water	4.65 ± 0.02	1.05 ± 0.03	ND	1.04 ± 0.02	3.06 ± 0.02

Site 6

Body	40.25± 0.03	32.64± 0.02	ND	5.50 ± 0.04	11.50 ± 0.10
Water	ND	2.70 ± 0.03	1.05 ± 0.02	1.99 ± 0.04	2.24 ± 0.03

Site 7

Body	7.0 ± 0.10	1.57 ± 0.10	1.25± 0.04	ND	7.50 ± 0.04
Water	ND	ND	ND	2.30 ± 0.02	2.45 ± 0.03

*ND- not detected

3.3.2.3. Lead

For Pb, the mean body concentrations were significantly higher ($p < 0.05$) than the water concentrations during winter 2000 (Table 31), while the reverse occurred during summer when the body concentrations were below detectable levels. This resulted in the water concentrations being significantly higher than the body concentrations ($p < 0.05$) at all other sites except site 5 and 7. During winter 2000, the mean body concentration at site 2 was 10 times higher than the mean water concentration, at site 6 the mean body concentration was 20 times higher than the water concentration, and at site 7 mean the body concentration was 13 times higher than the mean water concentration.

3.3.2.4. Zinc

For Zn, the mean body concentrations were significantly higher ($p < 0.001$) compared to those of ambient water during all five seasons (Table 32). During winter 2000, the mean body concentration at site 6 was as high as 48 times, and at site 7 as high as 36 times that of the water concentration. During spring, the body concentration at site 6 was 16 times as high as the water concentration, while at site 7 the body concentration was as high as 74 times the order of magnitude of water concentration.

Table 31: A comparison of Pb concentrations (± SE) in water (µg/L) and barnacle body samples (µg/g) (Site 1- Strand; site 2- Gordon's Bay; site 3- Glencairn; site 4- Muizenberg; site 5- Rooiels; site 6- Kleinmond; site 7- Miller's Point)

Site 1

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Body	3.13 ± 0.03	0.67 ± 0.04	ND	16.41± 0.04	20.47± 0.10
Water	0.14 ± 0.03	0.15 ± 0.02	1.70 ± 0.02	2.20± 0.02	14.0 ± 0.10

Site 2

Body	5.10 ± 0.10	ND	ND	0.57 ± 0.10	2.80 ± 0.04
Water	0.50 ± 0.04	0.35 ±	1.05 ± 0.02	2.84 ± 0.02	6.30 ± 0.02

Site 3

Body	1.96 ± 0.04	ND	ND	12.36± 0.10	15.33± 0.04
Water	1.15 ± 0.02	0.35 ± 0.02	4.80 ± 0.03	6.30 ± 0.02	9.20 ± 0.01

Site 4

Body	0.77 ± 0.04	ND	ND	9.62± 0.10	11.54± 0.03
Water	0.30 ± 0.02	10.17± 0.04	5.20 ± 0.03	4.30 ± 0.10	11.40± 0.02

Site 5

Body	3.33 ± 0.10	1.11 ± 0.01	0.56 ± 0.03	17.33± 0.03	20.0 ± 0.10
Water	1.65 ± 0.02	0.60 ± 0.02	4.65 ± 0.02	5.09 ± 0.03	10.30± 0.03

Site 6

Body	6.0± 0.04	ND	ND	1.50 ± 0.04	3.75 ± 0.03
Water	0.30 ± 0.02	0.30 ± 0.03	3.05 ± 0.02	3.85 ± 0.02	4.90 ± 0.03

Site 7

Body	9.0 ± 0.04	ND	12.60 ±0.04	18.75 ± 0.03	20.0 ± 0.10
Water	0.67 ± 0.01	0.04 ± 0.02	0.35 ± 0.02	3.40 ± 0.03	5.20 ± 0.03

*ND- not detected

Table 32: A comparison of Zn concentrations (± SE) in water and barnacle body samples (Site 1- Strand; site 2- Gordon's Bay; site 3- Glencairn; site 4- Muizenberg; site 5- Rooiels; site 6- Kleinmond; site 7- Miller's Point)

	Winter 2000	Spring	Summer	Autumn 2001	Winter 2001
	Site 1				
Body (µg/g)	57.97 ± 0.10	30.33 ± 0.03	43.75 ± 0.03	46.88 ± 0.10	65.63 ± 0.02
Water (µg/L)	3.35 ± 0.02	4.3 ± 0.03	9.95 ± 0.03	10.12± 0.04	12.44 ± 0.02
	Site 2				
Body (µg/g)	93.26 ± 0.03	38.85 ± 0.03	52.71 ± 0.02	65.24 ± 0.03	87.0 ± 0.04
Water (µg/L)	16.95 ± 0.02	12.3± 0.03	8.0 ± 0.10	10.95 ± 0.03	14.87 ± 0.02
	Site 3				
Body (µg/g)	69.22 ± 0.03	37.0 ± 0.10	27.55 ± 0.10	104.9 ± 0.03	127.45 ± 0.02
Water (µg/L)	24.6 ± 0.03	8.1 ± 0.02	16.3 ± 0.03	17.8 ± 0.04	20.3 ± 0.02
	Site 4				
Body (µg/g)	87.69 ± 0.03	64.29 ± 0.04	52.31 ± 0.10	144.81 ± 0.04	179.0 ± 0.10
Water (µg/L)	30.15 ± 0.03	9.3 ± 0.02	16.65 ± 0.10	17.0 ± 0.04	18.22 ± 0.02
	Site 5				
Body (µg/g)	66.94 ± 0.03	50.0 ± 0.10	62.22 ± 0.10	62.5 ± 0.03	68.9 ± 0.03
Water (µg/L)	48.05 ± 0.10	5.6 ± 0.02	5.13 ± 0.03	7.09 ± 0.02	10.5 ± 0.01
	Site 6				
Body (µg/g)	209.0 ± 0.10	145.28± 0.03	40.81 ± 0.10	160.0 ± 0.10	170.55 ± 0.10
Water (µg/L)	4.35 ± 0.02	8.85 ± 0.04	7.1 ± 0.02	9.4 ± 0.02	14.6 ± 0.10
	Site 7				
Body (µg/g)	160.05 ± 0.10	140.32 ± 0.03	36.6 ± 0.10	120.75 ± 0.10	138.25 ± 0.10
Water (µg/L)	4.5 ± 0.02	1.9 ± 0.02	3.55 ± 0.10	6.3 ± 0.03	11.97 ± 0.01

3.4. DISCUSSION

The heavy metals that were accumulated most by the barnacles were Cd, Cu and Zn (Tables 24, 25 & 28). These findings are in agreement with those of previous authors elsewhere (Beyer et al., 1996; Ruelas-Inzunza & Paez-Osuna, 1998) who found that barnacles were strong net accumulators of these three heavy metals. The accumulation of Cu and Zn was probably due to the fact that they are essential elements (Elliott et al., 1985). The results of the present study did not indicate any negative correlation between Cu and Zn, as was found in the barnacle *Elminius modestus* by Elliott et al. (1985), who found that the two heavy metals reacted antagonistically in this species.

Of all the heavy metals analysed in the barnacle body, Zn occurred in the highest concentrations during all seasons. Possible sources of the Cu and Zn contamination in the present study could have been the boating and docking activities and anti-fouling paints on boats in the fishing and yachting harbours at sites 6 and 7 (Ruelas-Inzunza & Paez-Osuna, 1998). According to Rainbow (1997), barnacles accumulate Zn to very high body concentrations, storing it in pyrophosphate granules. The high Zn content in barnacles may be attributed to inorganic granules that contain up to 38% Zn and which accumulate in the tissues surrounding their midguts (Eisler, 1981). Barnacles move large volumes of water across the permeable sites of their cirri during feeding and respiration (Rainbow et al. 1990), which may lead to an increased uptake of metal-rich plankton and detritus matter. Previous studies elsewhere (Rainbow & White, 1989; Rainbow et al., 1990; Beyer et al., 1996; Reish et al., 1999) recorded very high Zn levels of up to 150 000 µg/g dry weight being accumulated by barnacles.

The barnacle body concentrations of heavy metals were many times higher than those of the surrounding water, although the highest body concentrations did not always correspond to the maximum water concentrations (Tables 29 – 31). This is in agreement with the findings of other authors elsewhere (Sarki et al., 1995; Deacon & Driver, 1999), who found that accumulation could be up to and more than three orders of magnitude higher than in the aqueous phase.

In the present study, the water samples were taken right from the shoreline, where the water can be expected to be continually subjected to considerable variation in metal concentration within a short time, as found in other coastal regions by Shiber & Shatila (1978), which may explain the variability in concentrations of the external environment.

Enhanced accumulation of Cd at low salinity is widespread in marine organisms, including crustaceans (Powell & White, 1990; Blackmore, 1999), which may have contributed to the higher Cd body concentrations in the barnacles at site 5 (Table 9, Chapter 2; & Table 28), which is subjected to freshwater inflows via the Rooiels

River mouth. According to Rainbow (1997), Cd in seawater exists as chloro-complexes $CdCl^+$ and $CdCl^-_3$. Reduced salinity decreases the chloride concentration of the ambient environment, with an automatic reduction in chloro-complexation and a corresponding increase in the rate of uptake of Cd (Rainbow, 1997). Cd accumulation by barnacles was found, therefore, to be a function of free Cd^{2+} ions which are available in solution (Eisler, 1981). Since barnacles are sessile organisms, the accumulated heavy metal levels in their bodies represent integrated contamination levels over time (Schulz-Baldes, 1977). Although the SOMCHEM weapons testing site in the catchment area of site 5 was closed down in 1994 (Cock & McKenzie, 1998), it could be speculated that the barnacles at this site, due to their sessile nature, may have accumulated the contaminants into their bodies over time, resulting in the significantly high concentrations of Cd (Tables 24 & 29). Heavy metals which could have accumulated in the soil at the weapons testing site may still be leaching out into the runoff entering the ocean at the site, hence their continued detection at this site. No evidence of the increased presence of either cadmium or any other heavy metals were available to confirm this.

In the present study, significantly higher heavy metal body concentrations (Cu, Ni, Pb and Zn) were measured during winter compared to the other seasons. It was suggested previously that higher summer concentrations in the bodies of aquatic organisms may be related to the increase in phytoplankton productivity and increased amount of diatoms in other regions (Ireland, 1974), which would be expected to increase the metals available from ingested food. The higher concentrations measured during winter in the present study may also be related to increased heavy metal concentrations in the water as a result of high run-off during the winter rainy season in the area (Taljaard et al., 2000). The significantly higher rainfall that was recorded during the winter months (Table 4, Chapter 2) may have resulted in the mobilization of pollutants from diffuse sources, as reflected in the elevated winter concentrations.

3.5. CONCLUSION

The concentrations of Cd, Cu, Pb and Zn in *T. serrata* varied according to the time of sampling and collection site. Seasonal variations in the body weight, which are linked to the reproductive cycle, as well as changes in food availability, may have influenced heavy metal body loads. The ability of barnacles to accumulate heavy metals, as demonstrated by the results of the present study, make them potential biomonitors of heavy metal contamination in the intertidal zone. It may be concluded that the barnacles *T. serrata* are suitable biomonitors for Cd, Cu and Zn.

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CHAPTER 4 – COMPARATIVE ASSESSMENT OF HEAVY METAL LEVELS IN THE PERIWINKLES *OXYSTELE TIGRINA AND O. SINENSIS* (MOLLUSCA: GASTROPODA) FROM FALSE BAY

4.1. INTRODUCTION

Aquatic organisms are able to accumulate a whole range of pollutants into their soft tissues, and can therefore be used as sentinel organisms in pollution monitoring programmes (Giamberini & Pihan, 1997). The bioaccumulation of contaminants in aquatic organisms depends on extrinsic physico-chemical factors such as salinity, temperature and pH, the concentration of the chemical in the ambient environment, the food source, the position of the organism in the food chain, as well as its metabolism (Rainbow et al., 1990). According to Rainbow (1995), the major routes of uptake of contaminants depend on the dietary lifestyle of each species, that is, via the sediments for bottom feeders, and via suspended particles and water column for filter feeders.

Gastropods have not been studied as extensively as bivalves as potential biomonitors (Burkhart & Dallinger, 1993). In view of the successful use of periwinkles in determining metal contamination effects in European situations (Kang et al., 1999), it seemed of importance to assess whether the False Bay species could be similarly employed. The aim of this part of the study, therefore, was to determine whether the two *Oxystele* species found in the False Bay intertidal zone, and occurring over a wide range along the South African coast, could be used as biomonitors of heavy metal contamination. The aim was also to assess whether the shells of these organisms can be used to monitor heavy metal pollution. Comparative studies of heavy metals in both the soft tissues and shells were undertaken. The *Oxystele* species were chosen because of their abundance at three of the sampling sites in False Bay (Branch, 1974), and the fact that, as herbivorous grazers of algae, they represent a different feeding habit to that of filter feeders commonly used in biomonitoring studies.

4.2. MATERIALS AND METHODS

4.2.1. Animal sampling

Fifty individuals of the species *O. tigrina* and *O. sinensis* were collected during three seasons, namely, winter, spring and summer of the year 2000. The animal specimens were collected from the three sites where the two species co-exist in the study area (sites 1, 3 and 7) (Figure 1, Chapter 2). The specimens were collected by hand from the rocky shore, during low tide. The specimens were then placed in plastic buckets containing site water for transporting to the laboratory. In the laboratory, each individual was dried with a paper towel and weighed, and the shell length measured using callipers. The specimens were put in plastic bags and frozen at -20^0 C for storage until further analysis.

4.2.2. Heavy metal analysis

After the thawing of all samples, the soft tissues were separated from the shells, and then the two tissue types were pooled separately and oven-dried at 60^0 C for 48h. The samples were homogenised by grinding with pestle and mortar, and then acid-digested as described previously (p. 18). The concentrations of Cd, Cu, Ni, Pb and Zn were determined by AAS.

4.2.3. Statistical analysis

The differences between the soft tissue and shell concentrations were determined by using t-tests. One-way ANOVA was used to determine the role of site and season on the heavy metal concentration in each species.

4.3. RESULTS

4.3.1. Cadmium concentrations in soft tissues and shells

Table 33 shows the mean Cd concentrations which were measured during winter 2000 in the soft tissues and shells of *O. tigrina* and *O. sinensis*. The mean concentrations in the soft tissues (32.00 ± 010 µg/g dry weight) and shells (19.00 ± 0.10 µg/g) of *O. tigrina* collected from site 7 were significantly higher than those of *O. sinensis* ($p < 0.05$, n = 5 pool of 50 samples).

Table 33: Cd concentrations (µg/g ± SE, n = 5) in the soft tissues and shells of *Oxystele* species measured at three sites during winter 2000 (Site 1- Strand; 3- Glencairn; 7- Miller's Point)

	Soft	Shell
Site 1		
O. tigrina	5.0 ± 0.04	1.24 ± 0.03
O. sinensis	4.0 ± 0.10	2.83 ± 0.10
Site 3		
O. tigrina	6.45 ± 0.10	0.80 ± 0.04
O. sinensis	1.25 ± 0.10	1.26 ± 0.10
Site 7		
O. tigrina	32.0 ± 0.10	19.0 ± 0.10
O. sinensis	5.0 ± 0.04	0.10 ± 0.10

A comparison of the soft tissue and shell concentrations of Cd of each species was carried out using t-tests. The results showed significantly higher Cd concentrations in the soft tissues at sites 3 and 7 for *O. tigrina* ($p < 0.001$), and at sites 1 and 7 for *O. sinensis* ($p < 0.001$).

During spring 2000 (Table 34), the mean Cd concentrations measured in the soft tissues of the two species were more or less similar at site 3, while the mean concentrations of their shells were more or less similar at sites 1 and 7. The highest soft tissue mean concentration in *O. sinensis* (17.24 ± 0.03 µg/g) was measured at site 7. The shell concentrations measured at this site ranged between 0.57 (± 0.04) and 3.33 (± 0.10) µg/g in *O. tigrina*, and between 0.27 (± 0.10) and 1.00 (± 0. 0.10) µg/g in *O. sinensis.*

Table 34: Cd concentrations (µg/g ± SE, n = 5) in soft tissues and shells of *Oxystele* species measured at the three sites during spring 2000 (Site 1-Strand; 3-Glencairn; 7-Miller's Point)

	Soft	Shell
Site 1		
O. tigrina	4.35 ± 0.10	0.80 ± 0.10
O. sinensis	1.33 ± 0.04	1.0 ± 0.10
Site 3		
O. tigrina	4.38 ± 0.10	3.33 ± 0.10
O. sinensis	4.67 ± 0.10	0.67 ± 0.10
Site 7		
O. tigrina	2.22 ± 0.10	0.57 ± 0.10
O. sinensis	17.24 ± 0.03	0.27 ± 0.10

The t-tests indicated no significant differences between the mean Cd concentrations of soft tissues of the two species ($p > 0.05$), and between the shell samples ($p > 0.05$, $n = 5$) during spring 2000. The soft tissue concentrations of Cd in *O. tigrina* were significantly higher than those of the shells at all three sites ($p < 0.001$), while for *O. sinensis*, significant differences were found between the two body compartments at sites 3 and 7 ($p< 0.001$).

During summer 2000, the soft tissue mean Cd concentrations (Table 35) of *O. tigrina* were significantly higher ($p >< 0.05$) than those of *O. sinensis* at sites 1 and 7, while at site 3, the soft tissue mean concentrations of *O. sinensis* was significantly higher than those of *O. tigrina* ($p < 0.05$). No Cd was detected in the *O. sinensis* shells.

Table 35: Cd concentrations (µg/g ± SE, n = 5) in soft tissues and shells of *Oxystele* species measured at the three sites during summer 2000 (Site 1-Strand; 3-Glencairn; 7-Miller's Point)

	Soft	Shell
Site 1		
O. tigrina	0.51 ± 0.04	0.30 ± 0.04
O. sinensis	0.30 ± 0.04	ND
Site 3		
O. tigrina	0.95 ± 0.10	1.50 ± 0.10
O. sinensis	1.75 ± 0.04	ND
Site 7		
O. tigrina	1.88 ± 0.10	0.95 ± 0.04
O. sinensis	0.70 ± 0.04	ND

*ND- not detected

4.3.2. *Copper*

The mean Cu concentrations (Table 36) measured during winter 2000 in the soft tissues of *O. tigrina* ranged between 29.00 (± 0.10) and 70.25 (± 0.04) µg/g, while those of *O. sinensis* ranged between 19.20 (± 0.03) and 28.75 (± 0.10) µg/g. The highest mean concentration in the soft tissues of *O. tigrina* was measured in the samples from site 1,while for *O. sinensis*, the highest mean concentration was measured at site 3. In the shell samples, the mean Cu concentrations of the two species were more or less similar at site 1, while Cu was not detected in the shell samples of both species obtained from site 7. The t-tests indicated significant differences between *O. tigrina* and *O. sinensis* mean Cu concentrations of the soft tissues ($p < 0.001$, n = 5 pool of 50 samples) at sites 1 and 3. For the shell samples, the mean Cu concentrations of *O. sinensis* from site 3 were significantly higher than those of *O. tigrina* ($p < 0.001$) during this period.

Table 36: Cu concentrations (µg/g ± SE, n = 5) in soft tissues and shells of *Oxystele* species measured at the three sites during winter 2000 (Site 1-Strand; 3-Glencairn; 7-Miller's Point)

	Soft	Shell
Site 1		
O. tigrina	70.25 ± 0.04	3.0 ± 0.10
O. sinensis	19.20 ± 0.03	3.50 ± 0.10
Site 3		
O. tigrina	56.61 ± 0.04	1.58 ± 0.04
O. sinensis	28.75 ± 0.10	27.13 ± 0.11
Site 7		
O. tigrina	29.0 ± 0.10	ND
O. sinensis	20.0 ± 0.10	ND

*ND- not detected

During spring, the mean Cu concentrations (Table 37) in the soft tissues of *O. tigrina* ranged between 14.79 (± 0.04) and 43.59 (± 0.10) µg/g dry weight, with the highest concentration being measured in the samples from site 1. The mean Cu concentrations of *O. sinensis* during this season ranged between 14.00 (± 0.10) and 21.00 (± 0.10) µg/g, with the highest being measured at site 3. The mean Cu shell concentrations of *O. tigrina* ranged between undetectable levels and 5.89 (± 0.04) µg/g, while no Cu was detected in the shell samples of *O. sinensis.*

Table 37: Cu concentrations (µg/g ± SE) in soft tissues and shells of *Oxystele* species measured at the three sites during spring 2000 (Site 1-Strand; 3-Glencairn; 7-Miller's Point) (OT- *O. tigrina;* OS- *O. sinensis*)

	Soft	Shell
Site 1		
O. tigrina	43.59 ± 0.10	5.89 ± 0.04
O. sinensis	15.33 ± 0.10	ND
Site 3		
O. tigrina	30.67 ± 0.04	3.13 ± 0.10
O. sinensis	21.0 ± 0.10	ND
Site 7		
O. tigrina	14.79 ± 0.04	ND
O. sinensis	14.0 ± 0.04	ND

*ND- not detected

During summer 2000, the mean Cu concentrations in the soft tissues of *O. tigrina* (Table 38) ranged between 8.89 (± 0.03) and 25.23 (± 0 10) µg/g, while those in the soft tissues of *O. sinensis* ranged from undetectable levels to 12.00 (± 0.10) µg/g. In the shell samples, Cu was detected only at site 1 in *O. tigrina* samples, while none was detected in any of the *O. sinensis* samples.

The t-tests indicated significant differences between the two species regarding their mean Cu soft tissue concentrations at site 1 ($p < 0.05$, $n = 5$). The shell concentrations of the samples of *O. tigrina* from sites 1 and 3 were significantly higher than those of *O. sinensis* ($p < 0.05$) during this period. The t-tests also showed that the soft tissue mean Cu concentrations of both species were significantly higher than those of the shells at all sites ($p < 0.001$).

Table 38: Cu concentrations (µg/g ± SE, n = 5) in soft tissues and shells of *Oxystele* species measured at the three sites during summer 2000 (Site 1-Strand; 3-Glencairn; 7-Miller's Point) (OT- *O. tigrina;* OS- *O. sinensis)*

	Soft	Shell
Site 1		
O. tigrina	25.23 ± 0.10	2.77 ± 0.04
O. sinensis	ND	ND
Site 3		
O. tigrina	15.24 ± 0.04	ND
O. sinensis	ND	ND
Site 7		
O. tigrina	8.89 ± 0.03	ND
O. sinensis	12.0 ± 0.10	ND

*ND- not detected

The t-tests indicated significant differences between the two species regarding the mean Cu concentrations in the soft tissues samples obtained from sites 1 and 3 ($p < 0.001$), as well as those of the shell samples from site 1 ($p < 0.05$, $n = 5$) during summer. Also, the soft tissues of O. tigrina had significantly higher mean Cu concentrations than the shells at all three sites during summer ($p < 001$, $n = 5$).

One-way ANOVA indicated no significant seasonal or spatial differences in the mean Cu concentrations of the soft tissues of *O. tigrina* ($p > 0.05$, $n = 5$), and no significant seasonal or spatial differences in the mean Cu concentrations of its shell samples ($p > 0.05$, $n = 5$). For *O. sinensis,* there were significant seasonal variations in the mean Cu concentrations of soft tissues, with the winter 2000 concentrations being significantly higher than those measured during the other two seasons ($p < 0.05$, $n = 5$). There were, however, no significant seasonal differences in the mean Cu concentrations of its shell samples ($p > 0.05$, $n = 5$). There were also no significant spatial variations in the mean Cu concentrations of its soft tissues ($p > 0.5$), or of its shell samples ($p > 0.5$).

4.3.3. *Nickel*

The winter concentrations of Ni (Table 39) in the soft tissues of both species were in the order: site 1 > site 3 > site 7, being undetected in all the samples from site 7 (Figure 53). The shell concentrations ranged between undetectable levels and 1.00 (± 0.10) µg/g for *O. tigrina,* and between undetectable levels and 0.27 (± 0.11) µg/g for *O. sinensis.* The soft tissues of *O. tigrina* had significantly higher mean Ni concentrations than the shells at sites 1 and 3 ($p < 0.001$), while for *O. sinensis,* the soft tissues had significantly higher mean Ni concentrations than the shells at site 1 only ($p < 0.05$, $n = 5$ pool of 50 samples).

Table 39: Ni concentrations (µg/g ± SE) in soft tissues and shells of *Oxystele* species measured at the three sites during winter 2000 (Site 1-Strand; 3-Glencarin; 7-Miller's Point)

	Soft	Shell
Site 1		
O. tigrina	6.50 ± 0.04	1.0 ± 0.10
O. sinensis	2.0 ± 0.14	0.20 ± 0.11
Site 3		
O. tigrina	1.36 ± 0.04	0.07 ± 0.03
O. sinensis	0.63 ± 0.10	0.27 ± 0.11
Site 7		
O. tigrina	ND	ND
O. sinensis	ND	ND

*ND- not detected

During spring, the soft tissue samples of *O. tigrina* which contained some Ni were those from site 1 (0.87 ± 0.10 µg/g) and site 3 (0.90 ± 0.04 µg/g), while those of *O. tigrina* samples from site 7 were below detectable levels. For *O. sinensis*, Ni was detected in the soft tissue samples from site 3 only (1.33 ± 0.10 µg/g). Ni was not detected in any of the shell samples of both species from the different sites.

During summer, only the soft tissue samples of *O. tigrina* from site 1 were found to contain Ni (0.74 ± 0.03 μg/g), while the Ni concentrations of all the other soft tissue and shell samples were below detectable levels. There were no significant differences between the mean Ni concentrations of the two species during summer ($p > 0.05$), for both the soft tissues and the shell samples. One-way ANOVA showed that there were no significant seasonal or spatial variations in the mean Ni concentrations of each species, for both the soft tissues and shell samples ($p > 0.05$).

4.3.4. *Lead*

The mean Pb concentrations obtained during winter 2000 are shown in Table 40. The soft tissue concentrations of *O. tigrina* were in the order: site 1 > site 3 > site 7. No Pb was detected in *O. sinensis* soft tissue samples obtained from sites 3 and 7. The shell mean Pb concentrations of *O. tigrina* ranged between 1.00 (± 0.04) and 6.20 (± 0.04) μg/g, while those of *O. sinensis* ranged between undetectable levels and 1.88 (± 0.14) μg/g. There were no significant differences between *O. tigrina* and *O. sinensis* mean Pb concentrations of the soft tissue and shell samples ($p > 0.5$) during winter 2000.

Table 40: Pb concentrations (μg/g ± SE) in soft tissues and shells of *Oxystele* species measured at three sites during winter 2000 (Site 1-Strand; 3-Glencairn; 7-Miller's Point)

	Soft	Shell
Site 1		
O. tigrina	3.0 ± 0.10	6.20 ± 0.04
O. sinensis	0.06 ± 0.14	0.58 ± 0.13
Site 3		
O. tigrina	2.91 ± 0.04	01.20 ± 0.04
O. sinensis	ND	1.88 ± 0.10
Site 7		
O. tigrina	1.0 ± 0.04	1.0 ± 0.03
O. sinensis	ND	ND

*ND- not detected

The t-tests showed significantly higher mean Pb concentrations in the shells of *O. tigrina* from site 1 compared to the soft tissues ($p < 0.05, n = 5$), as well as a significant difference between the means of the soft tissue and shell concentrations of Pb in *O. sinensis* samples from sites 1 and 3 ($p < 0.05$).

The Pb concentrations measured in the soft tissue samples of the *O. tigrina* and *O. sinensis* from site 1 during spring were more or less similar (4.35 ± 0.03 and 4.0 ± 0.10 respectively), while no Pb was detected in any of the shell samples. There were no significant differences between the two species regarding their mean Pb concentrations in the soft tissues and shells during spring ($p > 0.5$). The summer mean Pb concentrations in the soft tissues of *O. tigrina* were all below detectable levels (Table 41), while those of *O. sinensis* were detected at sites 1 and 3, but not at site 7. No Pb was detected in the shell samples of *O. tigrina*, while in those of *O. sinensis*, Pb was detected at sites 1 and 3, but not at site 7. The t-tests showed that *O. sinensis* had significantly higher mean Pb concentrations compared to *O. tigrina* at sites 1 and 3 ($p < 0.001$) during summer. One-way ANOVA showed that there were no significant seasonal or spatial variations in the mean Pb concentrations of each species, for both the soft tissue and shell samples ($p > 0.5$).

4.3.5. *Zinc*

The winter mean Zn concentrations in the soft tissues of *O. tigrina* ranged between 114.00 (± 0.20) and 195.00 (± 0.10) µg/g dry weight, while those measured in the soft tissues of *O. sinensis* ranged between 47.00 (± 0.45) and 98.40 (± 0.12) µg/g (Table 42). The soft tissue concentrations of both species decreased progressively from site 1 to site 7. The shell concentrations of the two species were more or less similar at site 1, while at site 7 the mean shell concentration of *O. tigrina* was higher than that of *O. sinensis*. The t-tests showed significant differences between the soft tissue concentrations of the two species at all three sites during winter 2000 ($p < 0.05$). The soft tissue concentrations of each species were significantly higher than the shell concentrations at all three sites during this period ($p < 0.001, n = 5$).

Table 41: Pb concentrations (µg/g ± SE, n = 5) in soft tissues and shells of *Oxystele* species measured at three sites during summer 2000 (Site 1-Strand; 3-Glencairn; 7-Miller's Point)

	Soft	Shell
Site 1		
O. tigrina	ND	ND
O. sinensis	2.0 ± 0.14	0.30 ± 0.12
Site 3		
O. tigrina	ND	ND
O. sinensis	0.40 ± 0.11	0.30 ± 0.10
Site 7		
O. tigrina	ND	ND
O. sinensis	ND	ND

*ND- not detected

Table 42: Zn concentrations (µg/g ± SE, n = 5) in soft tissues and shells of Oxystele measured at three sites during winter 2000 (Site 1-Strand; 3-Glencairn; 7-Miller's Point)

	Soft	Shell
Site 1		
O. tigrina	195 ± 0.10	56.0 ± 0.10
O. sinensis	98.40 ± 0.12	54.0 ± 0.13
Site 3		
O. tigrina	128.55 ± 0.80	37.50 ± 0.10
O. sinensis	87.50 ± 0.13	26.50 ± 0.11
Site 7		
O. tigrina	114.0 ± 0.20	66.0 ± 0.10
O. sinensis	47.0 ± 0.45	36.0 ± 0.14

The mean Zn concentrations measured during spring are shown in Table 43. The shell concentrations of *O. tigrina* were between 20.50 (± 0.04) and 55.79 (± 0.10) µg/g, and were significantly higher than those of *O. sinensis* at site 3 ($p < 0.001$). The mean Zn concentrations of the shells of *O. sinensis* were between 13.33 (± 0.10) and 51.00 (± 0.15) µg/g, and were significantly higher than those of *O. tigrina* at site 1 ($p < 0.001$). The t-tests showed significant differences between the two species regarding their soft tissue concentrations at sites 1 and 7 ($p < 0.05$, n = 5 pool of 50 samples). The soft tissue mean Zn concentrations were significantly higher than those of the shells ($p < 0.05$) at all sites for *O. tigrina,* and at site 7 for *O. sinensis.*

Table 43: Zn concentrations (µg/g ± SE, n = 5) in soft tissues and shells of *Oxystele* measured at the three sites during spring 2000 (Site 1-Strand; 3-Glencairn; 7-Miller's Point)

	Soft	Shell
Site 1		
O. tigrina	86.96 ± 0.04	20.50 ± 0.04
O. sinensis	53.0 ± 0.13	51.0 ± 0.15
Site 3		
O. tigrina	78.0 ± 0.10	55.79 ± 0.10
O. sinensis	61.53 ± 0.11	19.33 ± 0.10
Site 7		
O. tigrina	43.16 ± 0.03	22.86 ± 0.04
O. sinensis	16.55 ± 0.11	13.33 ± 0.10

During summer, the highest mean Zn concentrations measured in the soft tissues and shells of *O. tigrina* were those found at site 3 (Table 44). The soft tissues of *O. sinensis* obtained from site 7 had the highest mean Zn concentrations, while the shell samples from site 1 had the highest Zn concentrations. The t-tests showed that there were significant differences between the two species regarding their soft tissue concentrations at all three sites ($p < 0.5$), as well as their shell concentrations of Zn ($p < 0.5$) during summer. The soft tissues had significantly higher Zn concentrations than the shells for each species at the different sites ($p < 0.001$, $n = 5$).

Table 44: Zn concentrations (µg/g ± SE, n = 5) in soft tissues and shells of *Oxystele* species measured at three different sites during summer 2000 (Site 1-Strand; 3-Glencairn; 7-Miller's Point)

	Soft	Shell
Site 1		
O. tigrina	38.21 ± 0.03	1.83 ± 0.03
O. sinensis	79.90 ± 0.14	60.80 ± 0.10
Site 3		
O. tigrina	93.81 ± 0.03	116.0 ± 0.10
O. sinensis	23.0 ± 0.13	7.0 ± 0.14
Site 7		
O. tigrina	86.11 ± 0.10	25.91 ± 0.04
O. sinensis	115.0 ± 0.13	19.0 ± 0.19

One-way ANOVA showed that, for *O. tigrina*, there were no significant seasonal or spatial variations in the mean Zn concentrations of both the soft tissues and the shell samples ($p > 0.05$). For *O. sinensis*, there were no significant seasonal variations in the mean Zn concentrations of both the soft tissues and shells ($p > 0.05$). There were, however, significant spatial differences in the shell mean Zn concentrations, with those measured at site 1 being significantly higher than those from the other sites ($p < 0.05$).

4.4. DISCUSSION

The heavy metals Cd, Cu and Zn were detected in both the soft tissue and shell extracts of the two species, although the proportions of these heavy metals in the different body parts were sometimes significantly different between the species at each site. Generally, the two species accumulated more heavy metals into their soft tissues (63-100% for *O. tigrina;* 10-100% for *O. sinensis)* than into their shells (Tables 33-38). This was observed for the heavy metals Cd, Cu and Zn, with the exceptions being Ni and Pb, whose shell concentrations were either similar to, or slightly higher than those of the soft tissues.

According to Gundacker (2000), Pb is mainly accumulated in the exoskeleton of invertebrates, which may explain the presence of more Pb in the shell samples. Kinne (1984) suggested that the accumulation of heavy metals such as Cd and Pb in the shells was another route for detoxification, while others (Walsh et al., 1995) suggested that the shells act as a safe storage matrix for contaminants that are resistant to soft tissue detoxification mechanisms. According to Yasoshima & Takano (2001), organisms accumulate heavy metals within their crystalline calcitic shell structure, with those having calcareous skeletons such as shells, taking up Pb in their exoskeletons to a greater extent than those without them. The reason suggested for this is the fact that Pb follows similar biochemical pathways to Ca for which shelled animals have high assimilation efficiency (Walker et al., 1996). This may, therefore, account for the higher Pb concentrations observed in the shell samples compared to the soft tissues of the two species (Table 40). The accumulation of Cd by the two species may be related to the bioaccumulation of Cd by the phytoplankton and algae on which they feed, which may then be transferred to the gastropods in the next trophic level (Nassiri et al., 1997; O'Leary & Breen, 1997). According to the findings of Gundacker (2000), more Cd is accumulated in the digestive gland than in the exoskeleton, which may explain the higher Cd levels in the soft tissue extracts compared to those of the shells.

Cu and Zn were accumulated to high concentrations in the soft tissues of both species (Tables 36-38 & 42-44). These findings are in agreement with the results of previous studies for other gastropod species (Walker, 1977; Yasoshima & Takano, 2001). The accumulation of these heavy metals may be related to their nature as essential elements (Elliott et al., 1985). The high concentrations of Cu, especially in the samples from sites 1 and 3 (Tables 36-38) may also be related to the presence of the Cu-containing haemocyanin respiratory pigment that occurs in large amounts in the haemolymph of gastropods (O'Leary & Breen, 1997). Previous studies found Cu concentrations of 23 µg/g dry weight in *Ostrea edulis* from a clean area in Angseley, North Wales, and 221 µg/g in polluted Fal Estuary in Cornwall, England (George et al., 1978). In the mussel *Mytilus edulis* from a clean area at Paignton, South Devon, Cu levels of between 5.06 and 36.2 mg/kg were measured (Boalch et al., 1981). Kang et al. (1999) measured Cu levels ranging between 50 and 65 µg/g in *Mytilus* species collected from a contaminated area at Onsan Bay, while the periwinkle *Littorina brevicula* from the same area accumulated between 57 and 664 µg/g Cu. Certain metals, such as Ni and Pb, were not accumulated in significantly high concentrations, indicating either the absence of localised inputs, or the efficient physiological regulation of these metals by the organisms (Nicholson, 1999a).

The observed decreases in the mean concentrations of the heavy metals from winter to summer 2000 (Tables 35-44) may be related to the spawning peaks of *Oxystele*, which, according to Griffiths & Branch (1991) occur during February and September-October months for the False Bay species. Another reason for the lower spring-summer concentrations may be related to the effect of higher temperatures on the rate of excretion which increases during summer, thus leading to a detoxifying effect which in turn leads to decreases in the heavy metal concentrations in soft tissues. The higher winter concentrations may be related to the lower salinity values observed during winter (Table 9, Chapter 2), which may have caused increased heavy metal accumulation due to increased metal bioavailability (Hops, 1990).

Each species showed a different pattern of accumulation of the heavy metals in its soft tissues and shells, which may be due to the different detoxification pathways exhibited by each species, as found for other gastropods (Nicholson, 1999a). The seasonal variations observed for Cu and Zn in the two species may be due to the differences in the uptake rates, storage and release of heavy metals by the two species, as suggested by Frias-Espericueta et al. (1999) for other gastropods.

4.5. CONCLUSION

In the present study, the concentrations of heavy metals presented high variability depending on the metal and the type of tissue. Although the two species occurred within the same habitat, the concentrations of heavy metals in the soft tissue and shell fractions varied between the two species, which may be a reflection of species-specific accumulation, storage and detoxifying strategies. Heavy metal fluctuations in the environment were reflected in the seasonal and spatial variations observed for each species. The accumulation of high levels of Cd, Cu and Zn in the bodies of the periwinkles *O. tigrina* and *O. sinensis* may make them suitable candidates for the biomonitoring of these heavy metals. The accumulation of heavy metals in the shells of aquatic organisms suggests that shells may act as a storage matrix for environmental contaminants and can be used as valuable indicators of contamination. From the results of this part of the study, it seems that a simple relationship between the external environment and internal concentrations in organisms is often absent, and that even closely related species might show different patterns of accumulation of a particular metal.

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CHAPTER 5- HEAVY METAL CONCENTRATIONS IN THE SOFT TISSUES OF THE LIMPET *PATELLA OCULUS* (MOLLUSCA: PATELLIDAE) FROM FALSE BAY

5.1. INTRODUCTION

There are three measures of heavy metals in the marine environments, those being the water, sediments and biota concentrations (Rainbow, 1995). Measurements of dissolved metals in ambient water present a problem because they are typically low or below detection limits, and also vary widely over time (Rainbow, 1995). Water and sediment contaminant analyses do not enable the prediction of potential impacts on the biota (Ringwood et al., 1998). The use of biomonitors, therefore, has an advantage over chemical analyses (Phillips, 1976), since the former are able to accumulate heavy metals to high concentrations in their tissues, thus provide a time-integrated measure of bioavailable metals (Rainbow, 1995).

One way to identify contaminants and their effects on aquatic organisms is to examine the responses of biomonitors to environmental stress (Schuwerack et al., 2001). Mussels such as *Mytilus* species have been used widely as biomonitors of pollution (Ramelow, 1985). These organisms, however, are not found at all coastal locations. Another sessile organism, the limpet, has been proposed as a suitable biomonitor by previous authors (Shiber & Shatila, 1978; Ramelow, 1985). The limpet *Patella oculus* occurs abundantly along the False Bay coastline (Griffiths & Branch, 1991), but little is known about the relationship between the body and environmental heavy metal concentrations of limpets (Ramelow, 1985). Although not sessile, limpets have a limited feeding range, possibly making them suitable as biomonitors. The aim of this part of the study was to assess the levels of heavy metals Cd, Cu, Ni, Pb and Zn in the soft tissues of *P. oculus* to serve as a baseline reference, and to evaluate the utility of the limpet as a metal pollution biomonitor in the study area.

5.2. MATERIALS & METHODS

5.2.1. Animal sampling

Twenty individuals of similar shell length (4.0- 5.5 cm) were collected from the seven samplings sites (Figure 1, Chapter 2) during five seasons. The specimens were collected during low tide, and were dislodged from the rocky substrate using a stainless steel blade. The animals were placed in plastic buckets containing site water for transportation to the laboratory. At the laboratory, the animals were killed by freezing at -20^0 C overnight.

5.2.2. Heavy metal extraction and analysis

The frozen specimens were thawed, and the soft tissue material separated from the shells, pooled to form a composite sample, and then oven-dried at 60^0 C for 48h. The dried soft tissue material was homogenised by grinding with pestle and mortar, and aliquot samples (0.2- 0.3g) were placed in test tubes and digested with nitric and perchloric acid as described previously (p. 18). For this species, it was decided not to process the shells.

5.2.3. Statistical analysis

One-way ANOVA was used to determine the temporal and spatial variations in the heavy metal concentrations. The t-tests were used to compare soft tissue and water heavy metal concentrations. The Pearson correlation analyses were used to determine the relationships between the water parameters and soft tissue heavy metal concentrations, as well as the relationships between the water and soft tissue metal concentrations.

5.3. RESULTS

5.3.1. Heavy metal body loads measured during winter 2000

5.3.1.1. *Cadmium*

Table 45 shows the heavy metal concentrations in the soft tissue samples obtained during winter 2000. The soft tissue concentrations of Cd ranged between 6.00

(± 0.35) and 31.67 (± 0.10) µg/g dry weight, with the lowest concentration being measured at site 3 and the highest at site 1. One-way ANOVA showed that site 1 mean Cd concentration was significantly higher than those from the other sites ($p < 0.001$, n = 5 pool of 20 samples).

The soft tissue concentrations of Cd measured at the different sites during winter 2000 were compared to those of water samples measured at the same time using t-tests. The analyses indicated significant differences between the water and soft tissue mean Cd concentrations ($p < 0.05$), with the soft tissue means being significantly higher than those of the water samples at all the sites.

TABLE 45: Mean concentrations of heavy metals (µg/g ± SE) measured in the soft tissues of the limpet *P. oculus* during winter 2000 (n = 5 pool of 20 samples)

Sites	Cd	Cu	Ni	Pb	Zn
1	31.67 ± 0.10	4.29 ± 0.10	3.59 ± 0.10	1.67 ± 0.11	49.00 ±0.15
2	12.50 ± 0.10	13.79 ± 0.10	5.99 ± 0.13	9.24 ± 0.10	29.58 ± 0.10
3	1.38 ± 0.10	ND	1.38 ± 0.14	1.25 ± 0.10	26.88 ± 0.13
4	6.00 ± 0.35	15.50 ± 0.12	2.50 ± 0.12	7.00 ± 0.15	218.50 ± 0.11
5	13.23 ± 0.10	20.00 ± 0.16	ND	14.00 ± 0.14	205.10 ± 0.18
6	18.60 ± 0.10	7.86 ± 0.13	2.00 ± 0.14	9.00 ± 0.14	196.43 ± 0.10
7	20.00 ± 0.12	2.00 ± 0.10	ND	ND	30.67 ± 0.10

*ND- not detected; (Site 1- Strand; 2- Gordon's Bay; 3- Glencairn; 4- Muizenberg; 5- Rooiels; 6- Kleinmond; 7- Miller's Point)

5.3.1.2. *Copper*

For Cu (Table 45), the soft tissue concentrations of Cu ranged between undetectable levels and 20.00 (± 0.16) µg/g dry weight. The highest Cu concentrations were found in the soft tissue samples from site 5. The t-tests showed that there were significant differences between the water and soft tissue mean concentrations of Cu ($p < 0.05$, n = 5), with the soft tissue concentrations generally being higher than those in the water samples at all the other sites except at site 3, where the water concentrations became

significantly higher ($p < 0.001$) than those of the soft tissue samples during winter 2000.

5.3.1.3. *Nickel*

During winter 2000, the mean Ni concentrations in the soft tissues of the limpet (Table 45) ranged between undetectable levels and 5.99 (± 0.13) µg/g. Ni was not detected in samples obtained from site 5 and site 7, while the highest mean concentration was measured at site 2. One-way ANOVA showed that these intersite differences in the soft tissue concentrations were also highly significant ($p < 0.001$).

The t-tests indicated significant differences between the water and soft tissue mean concentrations of Ni during this period ($p < 0.05$) at most sites.The mean body concentrations were generally higher than those of the water samples at all the other sites except at site 3, where the mean water concentrations became significantly higher than the body concentrations ($p < 0.001$). There were no significant differences between the water and soft tissue concentrations at sites 5 and 7.

5.3.1.4. *Lead*

The mean Pb concentrations in the soft tissues were below detectable levels at site 7 (Table 45), while the highest mean concentration was also measured at site 5. One-way ANOVA indicated significant spatial differences in the mean Pb concentrations for both the water and the soft tissue samples ($p < 0.001$, $n = 5$ pool of 20 samples), with those obtained from site 5 being significantly higher than those from the other sites. The t-tests also indicated significant differences between the water and soft tissue mean concentrations of Pb during this season ($p < 0.05$), with the soft tissue concentrations being higher than those of the water samples at sites 1, 2, 4, 5 and 6. There was, however, no significant difference between the water and soft tissue mean Pb concentrations at site 3 ($p > 0.05$). At site 7, the water Pb concentrations were significantly higher than those found in the soft tissues ($p < 0.001$).

5.3.1.5. *Zinc*

The soft tissue mean concentrations of Zn during winter 2000 (Table 45) ranged between 26.88 (± 0.13) and 218.50 (± 0.11) µg/g, with significantly high concentrations ($p < 0.001$) being measured in the soft samples obtained from site 4. The t-tests showed that the soft tissue concentrations were significantly higher than those of the water at all sites, during this season ($p < 0.05$, $n = 5$). One-way ANOVA was used to compare the mean concentrations of the five heavy metals, in both the water and soft tissue samples. The results showed that Zn concentrations in both the water and soft tissues were significantly higher than the other four heavy metals ($p < 0.001$) at all the sites during winter 2000.

5.3.2. Heavy metal body loads measured during spring 2000

5.3.2.1. *Cadmium*

The heavy metal concentrations obtained in the soft tissues samples of the limpet during spring are shown in Table 46. The soft tissue mean Cd concentrations ranged between 6.00 (± 0.21) and 17.79 (± 0.10) µg/g dry weight. The lowest mean concentration was measured at site 4, while the highest was measured at site 6. One-way ANOVA showed that these intersite differences were highly significant ($p < 0.001$).

Table 46: Mean concentrations of heavy metals (µg/g ± SE, n = 5) measured during spring in the soft tissues of the limpet

	Cd	Cu	Ni	Pb	Zn
Site 1	7.08 ± 0.17	4.17 ± 0.10	24.79 ± 0.10	1.50 ± 0.10	27.08 ± 0.11
Site 2	9.04 ± 0.10	4.75 ± 0.13	16.54 ± 0.10	7.39 ± 0.10	98.0 ± 0.14
Site 3	8.80 ± 0.10	ND	ND	0.87 ± 0.10	18.55 ± 0.12
Site 4	6.0 ± 0.21	10.0 ± 0.15	4.0 ± 0.13	2.45 ± 0.10	210.0 ± 0.12
Site 5	12.93 ± 0.10	0.97 ± 0.11	8.83 ± 0.12	0.78 ± 0.10	162.42 ± 0.10
Site 6	17.79 ± 0.10	0.09 ± 0.10	12.80± 0.10	0.78 ± 0.12	113.23 ± 0.10
Site 7	15.0 ± 0.13	0.33 ± 0.10	ND	ND	26.15 ± 0.13

*ND- not detected; (Site 1-Strand; 2-Gordon's Bay; 3-Glencarin; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

The t-tests indicated significant differences occurred between the water and soft tissue mean concentrations of Cd during spring ($p < 0.05$), with the soft tissue mean Cd concentrations being higher than those of the water samples at all the sites.

5.3.2.2. Copper

The mean Cu concentrations measured at sites 1 and 2 (Table 46) were more or less similar. Cu was not detected in the tissue samples from site 3, while the highest mean concentration was obtained in the samples from site 4. One-way ANOVA indicated significant spatial variations in the mean Cu concentrations of the soft tissues ($p < 0.001$, $n = 5$ pool of 20 samples). The t-tests showed that the soft tissue concentrations were significantly higher than those of the water samples at sites 1, 2, 4 and 7 ($p < 0.001$). There were, however, no significant differences between the water and soft tissue mean concentrations of samples obtained from site 3 ($p > 0.05$), while at sites 5 and 6, the water concentrations were significantly higher than those of the soft tissues ($p < 0.001$).

5.3.2.3. *Nickel*

During spring, the soft tissue concentrations of Ni (Table 46) were below detection limits at sites 3 and 7, while the highest mean concentration was measured at site 1. One-way ANOVA showed that the Ni values differed significantly in the soft tissue samples from the different sites ($p < 0.001$, $n = 5$ pool of 20 samples). The t-tests indicated significant differences between the water and soft tissue mean concentrations of Ni during spring ($p < 0.05$), with the soft tissue concentrations being higher than those of the water samples at sites 1, 2, 4, 5 and 6. At sites 3 and 7, there were no significant differences between the water and soft tissue concentrations of Ni during spring ($p > 0.05$).

5.3.2.4. *Lead*

The mean Pb concentrations obtained in the soft tissue samples (Table 46) from sites 5 and 6 were the same. No Pb was detected in the soft tissue samples from site 7, while the highest Pb mean concentration in the soft tissues was obtained in samples from site 2. One-way ANOVA showed that these intersite differences were highly significant ($p < 0.001$, $n = 5$ pool of 20 samples). The t-tests showed that the soft tissue mean concentrations of Pb were significantly higher ($p < 0.001$) than those of the water samples obtained from all the other sites except those from sites 4 and 7. At these two sites, the water concentrations of Pb were significantly higher than those of the soft tissue samples ($p < 0.001$).

5.3.2.5. *Zinc*

The mean Zn concentrations measured during spring in the soft tissues ranged between 18.55 (± 0.12) and 210.00 (± 0.12) µg/g, with the highest mean value being measured at site 4 (Table 46). One-way ANOVA indicated significant spatial differences in the soft tissue mean Zn concentrations ($p < 0.001$, $n = 5$ pool of 20 samples) The t-tests showed that there were significant differences between the water and soft tissue mean concentrations of Zn during spring ($p < 0.05$), with those of the soft tissues being higher than those from water at all the sites. One-way ANOVA was used to compare the mean concentrations of the five heavy metals in the water and soft tissues during spring 2000. The results showed that in the water samples, the Zn concentrations were significantly higher ($p < 0.001$) than the other heavy metals at all other sites except site 4, where the Pb concentrations were significantly higher ($p < 0.001$) than the other heavy metals. In the soft tissues, the mean Zn concentrations were significantly higher than the other heavy metals at all the sites during spring ($p < 0.001$).

5.3.3. Heavy metal body loads measured during summer 2000

5.3.3.1. *Cadmium*

The mean Cd values measured in the soft tissues of the limpet during summer ranged between 0.69 (± 0.10 µg/g), which was measured at site 2, and 12.70 (± 0.10 µg/g), which was measured at site 7 (Table 47). One-way ANOVA showed that site 7 concentrations of Cd were significantly higher than those from other sites ($p < 0.001$). The t-tests showed that the mean soft tissue Cd concentrations were significantly higher ($p < 0.05$) than those of the water samples at all other sites except at site 2, where the water concentrations were significantly higher than those of the soft tissue concentrations ($p < 0.001$).

Table 47: Mean heavy metal concentrations (µg/g ± SE, n = 5) measured during summer in the soft tissues of the limpet

	Cd	Cu	Ni	Pb	Zn
Site 1	3.39 ± 0.13	ND	7.50 ± 0.11	3.04 ± 0.10	80.89 ± 0.10
Site 2	0.69 ± 0.10	1.55 ± 0.10	2.07 ± 0.13	1.55 ± 0.10	92.59 ± 0.15
Site 3	9.80± 0.12	ND	7.20 ± 0.12	1.20 ± 0.10	51.0 ± 0.13
Site 4	4.29 ± 0.10	4.82 ± 0.13	ND	0.16 ± 0.10	98.22 ± 0.10
Site 5	5.0 ± 0.14	0.20 ± 0.10	18.20 ± 0.12	6.0 ± 0.12	160.0 ± 0.17
Site 6	6.03 ± 0.16	0.24 ± 0.10	11.91± 0.14	9.52 ± 0.10	131.43 ± 0.10
Site 7	12.70 ± 0.10	0.21 ± 0.10	ND	ND	63.75 ± 0.13

*ND- not detected; (Site1- Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

5.3.3.2. *Copper*

Cu was not detected in the soft tissue samples from sites 1 and 3 (Table 47), while similar values of Cu were obtained at sites 5, 6 and 7. The highest mean concentration was measured in the soft tissue samples from site 4 One-way ANOVA showed that these spatial variations were highly significantly ($p < 0.001$).

The t-tests indicated significantly higher mean Cu concentrations in the soft tissues than in the water at sites 2, 4, 5 and 7 ($p < 0.001$), while at sites 1, 3 and 6, the water mean concentrations were significantly higher than those of the soft tissues ($p < 0.001$).

5.3.3.3. *Nickel*

The soft tissue mean Ni concentrations were below detection limits at sites 4 and 7 (Table 47), while similar values of Ni were measured in the soft tissue samples from sites 1 and 3. The highest mean concentration in the soft tissue samples was measured at site 5. One-way ANOVA indicated that the site 5 mean Ni concentration was significantly higher than that measured in the soft tissue samples from the other sites ($p < 0.001$). The t-tests showed that there were no significant differences between the water and soft tissue mean concentrations of Ni at site 7 ($p > 0.05$). The soft tissue mean concentrations of the samples obtained from sites 1, 5 and 6 were, however, significantly higher than those of the water samples ($p < 0.001$), while at sites 2, 3 and 4 the reverse occurred.

5.3.3.4. *Lead*

Pb was below detection limits at site 7 while the highest mean concentration was measured at site 6 (Table 47). One-way ANOVA showed that the mean Pb concentrations in the soft tissue samples obtained from site 6 were significantly higher than those obtained from the other sites ($p < 0.001$). The t-tests indicated significant differences between the water and soft tissue mean concentrations of Pb, with the soft tissue concentrations being significantly higher than those of the water samples at sites 1, 5 and 6 ($p < 0.001$). At sites 3, 4 and 7, the water concentrations were significantly higher than the soft tissue concentrations ($p < 0.001$).

5.3.3.5. *Zinc*

The lowest mean concentration of Zn in the soft tissues of the limpets was measured at site 3, while the highest mean concentration (160.00 ± 0.17 µg/g) was measured at site 5 (Table 47). One-way ANOVA showed that these intersite variations in the soft tissue concentrations were highly significant ($p < 0.001$).The t-tests indicated significant differences between the water and soft tissue mean concentrations ($p < 0.05$), with the soft tissue concentrations being higher than those in the water samples at all seven sites. One-way ANOVA was also used to compare the mean water and soft tissue concentrations of the different heavy metals during the summer season. The results showed that the mean Zn concentrations in both the water and soft tissue samples were significantly higher than those of the other heavy metals at all sites during summer ($p < 0.001$).

5.3.4. Heavy metals body loads measured during autumn 2001

5.3.4.1. *Cadmium*

The mean concentrations of heavy metals measured in the soft tissues of the limpet during autumn 2001 are shown in Table 48. The mean Cd concentrations measured at sites 1 and 2, and sites 3 and 4 were more or less similar, while the highest mean concentration was measured at site 6. One-way ANOVA showed that these intersite differences in the soft tissue concentrations of Cd were highly significant ($p < 0.001$). The t-tests showed that the soft tissue mean concentrations of Cd were significantly higher than those of the water samples ($p < 0.05$) at all other sites except at site 3, where the reverse occurred.

5.3.4.2. *Copper*

The mean Cu concentrations in the soft tissue samples were more or less similar at sites 5 and 6 (Table 48). The highest mean concentration was measured at site 2, while the lowest was measured at site 7. One-way ANOVA indicated significant spatial variations in the mean Cu concentrations in both the water ($p < 0.001$) and the soft tissue samples ($p < 0.001$). The t-tests indicated significant differences between the water and soft tissue mean concentrations of Cu ($p < 0.05$), with the soft tissue concentrations being higher than those in the water samples at all other

sites except at site 7. At site 7, the water concentrations of Cu were significantly higher than those of the soft tissue samples ($p < 0.001$).

TABLE 48: Mean concentrations of heavy metals (µg/g ± SE, n = 5) measured in the soft tissues of the limpet *P. oculus* during autumn 2001

Sites	Cd	Cu	Ni	Pb	Zn
1	7.29 ± 0.10	6.25 ± 0.13	9.58 ± 0 10	6.30 ± 0.10	91.47 ± 0.16
2	7.31 ± 0.10	11.50 ± 0.13	2.17 ± 0.14	3.68 ± 0.11	107.93 ± 0.13
3	5.18 ± 0.10	7.94 ± 0.17	13.62 ± 0.11	3.45 ± 0.10	83.62 ± 0.10
4	5.20 ± 0.10	8.00 ± 0.17	8.25 ± 0.12	5.75 ± 0.10	108.62 ± 0.12
5	23.25 ± 0.11	9.00 ± 0.10	26.25 ± 0.16	10.50 ± 0.12	182.50 ± 0.10
6	25.75 ± 0.10	9.50 ± 0.10	15.00 ± 0.16	10.25 ± 0.12	133.25 ± 0.13
7	14.99 ± 0.15	1.28 ± 0.10	ND	1.29 ± 0.10	72.90 ± 0.22

*ND- not detected (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rociels; 6-Kleinmond; 7-Miller's Point)

5.3.4.3. *Nickel*

Ni was not detected in the soft tissue samples from site 7, while reaching the highest value at site 5 (Table 48). One-way ANOVA showed that these spatial variations in the Ni concentrations of soft tissue samples were highly significant ($p < 0.001$). The t-tests indicated significant differences between the water and soft tissue mean concentrations of Ni, with those of the soft tissues being significantly higher than those of the water at sites 1, 3, 4, 5 and 6 ($p < 0.05$), during this season. At sites 2 and 7, the water concentrations of Ni were significantly higher than those of the soft tissues ($p < 0.001$).

5.3.4.4. *Lead*

The concentrations measured at the various sites ranged between 1.29 (± 0.10) and 10.50 (± 0.12) µg/g (Table 48), with lowest mean Pb concentration measured at site 7, while the highest mean concentration was measured at site 5. There were significant differences in the Pb concentrations of soft tissue samples obtained from the various sites ($p < 0.001$). The t-tests also indicated significant differences between the water (Appendix 10) and soft tissue mean concentrations of Pb during autumn 2001, with the soft tissue concentrations measured at sites 1, 2, 4, 5 and 6 being significantly

higher than those of the water samples ($p < 0.05$). At sites 3 and 7, however, the water concentrations were significantly higher than those of the soft tissues ($p < 0.001$).

5.3.4.5. *Zinc*

The mean Zn concentrations in the soft tissues (Table 48) ranged between 72.90 (± 0.22) and 182.50 (± 0.10) µg/g, which were measured at sites 7 and 5 respectively. One-way ANOVA showed that the mean Zn concentration measured in site 5 samples was significantly higher than those from the other sites ($p < 0.001$). The t-tests also showed that the soft tissue mean Zn concentrations were significantly higher than those of the water samples ($p < 0.05$) at all sites during autumn 2001.

One-way ANOVA was used to compare the heavy metal concentrations of the water and soft tissue samples during autumn 2001. The results showed that the mean Zn concentrations measured at all the sites were significantly higher than those of the other heavy metals in both the water and soft tissue samples during autumn ($p < 0.001$).

5.3.5. Heavy metal body loads measured during winter 2001

5.3.5.1. *Cadmium*

Table 49shows the heavy metal concentrations measured in the soft tissues during winter 2001. The mean Cd concentrations in the soft tissues ranged between 7.27 (± 0.10) and 31.50 (± 0.10), with the lowest mean concentrations being measured at site 3 and the highest at site 6. There were significant differences in the Cd concentrations of the soft tissue samples obtained from the various sites ($p < 0.001$). The t-tests showed that the soft tissue mean concentrations of Cd were significantly higher than those of water at all sites during this period ($p < 0.05$).

Table 49: Mean heavy metal concentrations (µg/g ± SE, n = 5) measured in the soft tissues of the limpet during winter 2001 (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

	Cd	Cu	Ni	Pb	Zn
Site 1	9.58 ± 0.10	8.38 ± 0.10	10.50 ± 0.10	7.92 ± 0.13	108.33 ± 0.17
Site 2	10.17 ± 0.03	10.99 ± 0.12	9.29 ± 0.13	4.72 ± 0.11	117.99 ± 0.13
Site 3	7.27 ± 0.10	8.15 ± 0.10	21.21 ± 0.12	4.55 ± 0 15	91.73 ± 0.10
Site 4	11.50 ± 0.14	11.24 ± 0.10	11.50 ± 0.12	6.50 ± 0.12	160.50 ± 0.12
Site 5	21.50 ± 0.12	11.57 ± 0.10	35.75 ± 0.12	12.75 ± 0.10	198.75± 0.14
Site 6	31.50 ± 0.10	10.59 ± 0.10	25.50± 0.10	11.50 ± 0.11	187.75 ± 0.16
Site 7	15.75 ± 0.10	5.25 ± 0.11	1.50 ± 0.11	3.50 ± 0.10	85.94 ± 0.15

5.3.5.2. *Copper*

The soft tissue mean Cu concentrations during winter 2001 ranged between 5.25 (± 0.11) and 11.57 (± 0.10) µg/g, which were measured at sites 7 and 5 respectively (Table 49). One-way ANOVA showed that the Cu concentrations measured in the soft tissue samples obtained from site 5 were significantly higher than those from the other sites ($p < 0.05$). The t-tests showed that the soft tissue mean concentrations of Cu measured during winter 2001 were significantly higher ($p < 0.001$) than those measured in the water at all the sites.

5.3.5.3. *Nickel*

The lowest Ni mean concentration in the soft tissues during winter 2001 was obtained from site 7, while (Table 49) the highest mean concentration was measured at site 5. One-way ANOVA indicated significant spatial variations in the soft tissue concentration ($p < 0.001$).

5.3.5.4. *Lead*

The mean concentrations of Pb in the soft tissues ranged between 3.50 (± 0.10) and 12.75 (± 0.10) µg/g, which were measured at sites 7 and 5 respectively (Table 49). One-way ANOVA showed that site 5 mean Pb concentrations were significantly higher than those from the other sites ($p < 0.001$). The t-tests showed that the water concentrations of Pb were significantly higher ($p < 0.001$) than those of the soft tissues at sites 1, 2, 3, 4 and 7, while at sites 5 and 6, the soft tissue concentrations were significantly higher than those of the water samples ($p < 0.001$).

5.3.5.5. *Zinc*

The mean Zn concentration in the soft tissues during winter 2001 ranged between 85.94 (± 0.15) and 198.75 (± 0.14) µg/g, with the lowest being measured at site 7 and highest at site 5 (Table 49). One-way ANOVA showed that these intersite variations were highly significant ($p < 0.001$). The t-tests indicated significant differences between the water and soft tissue concentrations ($p < 0.05$), with the soft tissue concentrations being significantly higher than those of the water samples at all the sites.

5.3.6. Comparison of present results with results from other studies

The results obtained for *Patella oculus* in the present study were compared to those from previous studies which were carried out in the south-west coast of South Africa (Darracott & Watling, 1975; Ramelow, 1985) (Table 50). The south-west coast is characterised by cool temperate climatic conditions (Griffiths & Branch, 1991) and subject to pollution loads from the Table Bay harbour and Saldanha Bay ore-loading operations (Bartlett & Pennig, 1982).

TABLE 50: Ranges of heavy metal concentrations obtained in *P. oculus* in the present study and elsewhere (Darracott & Watling, 1975*; Ramelow, 1985#) (µg/g dry weight)

Metal	Present study	Other studies
Cd	0.69 – 31.67	2.1# – 31.0*
Cu	ND – 20.00	3.5 – 13.7#
Ni	ND – 35.75	2.5* – 83.7#
Pb	ND – 14.00	0.3 – 10.4#
Zn	26.15 – 218.50	40.5*– 96.4#

5.4. DISCUSSION

The main factors that regulate the heavy metal concentrations in the invertebrate tissues are the concentrations in the surrounding environment, changes related to the food supply, the mechanism of uptake, storage and release, as well biological parameters such as the reproductive cycle (Frias-Espericueta et al., 1999). The results of the present study showed that the body concentrations of the various heavy metals were significantly higher than those of the water samples during most of the five seasons sampled, and at most of the sites (Tables 45- 49). This is in agreement with previous observations (Phillips, 1976; Lobel et al., 1982) that, in general, the metal concentrations in the tissues of marine biomonitors may be between 10^3 and 10^6 orders of magnitude higher than those in the surrounding water.

The accumulation of Cu, Cd, Pb and Zn into the soft tissues of *P. oculus* is in line with the findings of Boening (1999) that marine shellfish can accumulate high concentrations of these heavy metals into their soft tissues. *Patella* species have been found to accumulate Cu consistently, and have also been proposed as moderate indicators of Zn (Eisler, 1981).

In the present study, the limpet accumulated up to 20.00 µg/g Cu (Table 50) in its soft tissues, and it may be assumed that the presence of Cu in the limpets was related to the presence of a Cu-containing haemocyanin respiratory pigment which occurs in large amounts in the haemolymph of gastropod molluscs (O'Leary & Breen, 1997).

The presence of significantly high Pb concentrations in the water samples from site 5 during spring 2000 and winter 2001, compared to the other sites may be related to the high amount of road run-off that enters that sites via stormwater drains, especially during the high-precipitation winter period (Taljaard et al., 2000). Another source of Pb in the water may be the construction activities that have been taking place during the building of holiday homes at this site (Taljaard et al., 2000), in which Pb-based building materials in the form of roofing, anti-corrosive and wall-cladding materials may have been used, as suggested by the findings of Hutzinger (1982) elsewhere.

Salinity has been found to have a strong influence on the bioavailability and accumulation of heavy metals by molluscs (Cunningham, 1979). In the present study, the lowest water salinity values (Table 9, Chapter 2) were associated with the highest accumulation of Cd in the soft tissues of limpets collected from site 1 (Table 48), while the highest salinity at site 3 was associated with the lowest mean Cd concentration. This may be an indication of the influence of salinity on heavy metal uptake and accumulation by aquatic organisms, and is in agreement with the findings by previous authors (Coombs, 1979; Moore, 1981; Hops, 1990; Rainbow et al., 2000) that a reduction in salinity increases the uptake of Cd into the tissues of molluscs. The ability of limpets to accumulate Cd from their environment was demonstrated in previous studies which were carried out along the Bristol Channel (Shore et al., 1975).

Changes in temperature are known to trigger spawning in many molluscs (Branch, 1974). Branch (1974) has proposed a bi-annual spawning in *P. oculus* from the False Bay intertidal zone, which occurs during March or April (autumn), and September (spring). During this time, there is a progressive build-up of gonad weight until a sharp spawning occurs. In the present study, the significant decrease in the heavy

metal concentrations in the soft tissues during summer (Table 47) may be related to the metal reduction that accompanies the spawning process (Regoli, 1992). The increase in the metal concentrations of the soft tissues during the colder autumn and winter periods in the present study (Tables 48-50) may be related to the gonad maturation process that continues to occur while the ambient temperatures are decreasing (Branch, 1974).

The higher metal concentrations measured in the water samples and subsequent accumulations of high concentrations in the soft tissues during the winter period (Tables 45 & 49) in the present study may be related to the increased runoff rate resulting from the increased precipitation during the rainy winter period (Taljaard et al., 2000). The high amount of rainfall that was recorded in the study area during the winter months (Table 10, Chapter 2) supports this observation. The increased amount of runoff in the present study may have resulted in the mobilization of heavy metals from diffuse sources into the storm-water drains that eventually discharge into False Bay (Taljaard et al., 2000).

The heavy metal levels measured in the present study compare favourably with previous results obtained for *Patella* elsewhere (Table 50), except for the Zn concentrations which were higher in the present study. According to Frias-Espericueta et al. (1999), Zn is the key element in the reproductive cycle, which may explain the significantly higher concentrations of Zn during the winter period associated with gonad development of the limpet in the present study. According to Eisler (1981), Zn accumulation in molluscs is mediated by its interaction with calcium, iron and Cd. The limpets collected from site 5 tended to have high concentrations of Zn (Tables 45- 49), which may be related to their limited feeding range which may have made them vulnerable to long-term heavy metal contamination.

5.5. CONCLUSION

The results of this part of the study showed significant spatial and seasonal variations in the soft tissue metal concentrations. The variations in the body concentrations may be associated with factors such as the changes related to the reproductive cycle, and the changes in the ambient heavy metal concentrations. Salinity seemed to be an important factor in the accumulation of heavy metals by the soft tissues of the limpet. The heavy metal that was accumulated most in the soft tissues of the limpet was Zn, but this could be related to the reproductive cycle rather than environmental inputs. From the present results, it may be concluded that the limpet *Patella oculus* may be a suitable biomonitor of Cd and Pb.

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CHAPTER 6 – HEAVY METAL CONCENTRATIONS IN THE MUSSEL *CHOROMYTILUS MERIDIONALIS* (MOLLUSCA: BIVALVIA) FROM FALSE BAY

6.1. INTRODUCTION

One of the major concerns about the effects of contaminant inputs into coastal embayments is their build-up in the water and sediments, leading to the accumulation and adverse effects in the biota (Morse et al., 1993). The effects of pollutants on living organisms can be evaluated at different levels of body organization, that is, molecular, cellular, individual or population levels (Viarengo & Canesi, 1991). Marine bivalves are known to accumulate heavy metals to high concentrations without any apparent adverse effects (Regoli et al., 1991), thus making them suitable biomonitors of marine pollution. Mussels have been found to accumulate heavy metals by factors of between 10^2 and 10^5 (Cattani et al., 1999). The suitability of mussels as biomonitors is related to the fact that they are sedentary, filter-feeding organisms (Moore, 1985); they are often widespread and some species are tolerant of contaminant levels and fluctuations in the physico-chemical parameters (Connell et al., 1999), and some can even flourish in contaminated environments (Rainbow, 1995).

Investigations of metals in the soft tissues and shells of molluscs are an important aspect of environmental pollution monitoring (Szefer & Szefer, 1985). Mussels have played an important role in the development of biomonitoring programmes for heavy metals, especially *Mytilus* species in the Mussel Watch Programme in the United States of America (Rainbow, 1995). The mussel *Choromytilus meridionalis* commonly occurs in the False Bay intertidal zone, and is one of the mollusc species used in the South African marine-monitoring programme (Watling & Watling, 1976), hence its inclusion in the heavy metal assessment of False Bay in the present study. The aim of this part of the study was to determine the heavy metal concentrations in the soft tissues and shells of this species, as well as to assess whether there were any spatial or seasonal variations in the heavy metal levels. Care was taken not to confuse this species with another similar alien species, *Mytilus galloprovincialis,* and this was done by using the absence of a pitted resillal ridge as a feature which distinguishes

C. meridionalis from other bivalve species (Schurink & Griffiths, 1990), photographs of this species by Branch et al. (1994) and the fact that *C. meridionalis* inhabits the lower littoral zone which is subject to sand cover, unlike *M. galloprovincialis* which has very low silt-tolerance (Hammond, 2001).

6.2. MATERIALS AND METHODS

6.2.1. Animal sampling

Specimens of *C. meridionalis* were collected from the three sites (sites 4, 5 and 6) where they occur abundantly in the False Bay intertidal zone. Since body weight variations related to the biological state of the mussels may be a source of variability in the largest mussels (Bourgoin, 1990), this problem was circumvented by choosing mussels of a standard shell length, under the assumption that the mussels within a size class were of the same age. Thirty individuals of similar shell length (25- 50 mm) were sampled seasonally over a period of a year. It has been shown previously (Lobel et al., 1982) that the mussels within this shell-length range chosen were free of size effects. The animals were dislodged from the rocky shore during low tide using a stainless steel blade. After sampling, the specimens were transported to the laboratory in 5-L plastic buckets containing site water. At the laboratory, the animals were killed by freezing them at -20°C overnight.

6.2.2. Heavy metal extraction and analysis

The frozen animal samples were defrosted, and the soft tissues separated from the shells. The soft tissue and shell samples were pooled separately, and oven-dried at 60°C for 48h. The dried samples were then homogenized by grinding with pestle and mortar. Aliquot samples (0.2 – 0.3g) of the soft tissues and shells were then acid-digested with nitric and perchloric acid as described previously (Chapter 2, p. 18). The concentrations of Cd, Cu, Ni, Pb and Zn in the soft tissue and shell samples were determined using the AAS method which has been described earlier. The heavy metal concentrations of the soft tissues were determined for five seasons, while those of the shell samples were determined only for the first three seasons, that is, from winter 2000 to summer 2000.

6.2.3. Statistical analysis

One-way ANOVA was used to determine the seasonal and spatial variations in the heavy metal concentrations in the water, soft tissues and shells. The soft tissue and shell concentrations were compared by using the t-tests.

6.3. RESULTS

6.3.1. Heavy metal concentrations in the soft tissues of *C. meridionalis*

6.3.1.1. *Cadmium*

The mean concentrations of Cd measured in the soft tissues are shown in Table 51. The values ranged between 0.57 (± 0.10) and 16.25 (± 0.12) µg/g dry weight. Generally, the concentrations tended to decrease slightly from winter to spring 2000. At site 4, the concentrations increased progressively from summer through autumn to winter 2001, while at the two other sites the values increased from autumn to winter 2001. One-way ANOVA showed that these seasonal variations of Cd in the soft tissues were highly significant ($p < 0.001$, n = 5 pool of 30 samples).

Table 51: Cd concentrations measured in the soft tissue samples (µg/g ± SE, n = 5) of the mussel from the three sites during different seasons

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 4	2.78 ± 0.10	1.17 ± 0.10	8.09 ± 0.10	9.11 ± 0.11	9.84 ± 0.10
Site 5	16.25 ± 0.12	7.78 ± 0.10	3.0 ± 0.11	4.14 ± 0.10	6.10 ± 0.10
Site 6	3.69 ± 0.10	0.57 ± 0.10	0.67 ± 0.10	2.77 ± 0.10	3.09 ± 0.10

(Site 4- Muizenberg; 5- Rooiels; 6-Kleinmond)

6.3.1.2. *Copper*

Table 52 shows the mean concentrations of Cu in the soft tissues. The mean concentrations ranged between undetectable levels, which were measured at sites 5 and 6 during summer, and 5.00 (± 0.10) µg/g dry weight, which was measured at site 5 during winter 2000. The values obtained at the three sites decreased significantly ($p < 0.001$, n = 5 pool of 30 samples) from winter 2000 through spring to summer 2000, and then increased from autumn to winter 2001. There were also significant spatial differences in the mean Cu concentrations of the soft tissues ($p < 0.001$), with the concentrations measured at site 5 being significantly higher than those obtained at the other sites during winter 2000.

Table 52: Cu concentrations measured in the soft tissue samples (µg/g ± SE, n = 5) of the mussel from the three sites during different seasons

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 4	3.44 ± 0.10	1.20 ± 0.12	0.36 ± 0.10	1.49 ± 0.10	2.59 ± 0.10
Site 5	5.0 ± 0.10	1.0 ± 0.10	ND	0.48 ± 0.10	1.79 ± 0.10
Site 6	3.69 ± 0.04	0.57 ± 0.04	ND	0.94 ± 0.04	2.10 ± 0.10

*ND- not detected (Site 4-Muizenberg; 4- Rooiels; 5- Kleinmond)

6.3.1.3. *Nickel*

The mean Ni concentrations ranged between undetectable levels, measured at site 6 during summer, and 10.20 µg/g dry weight, which was measured at site 4 during winter 2001 (Table 53). One-way ANOVA indicated significant seasonal and spatial differences in the Ni concentrations measured in the soft tissues ($p < 0.001$). The Ni concentrations measured at site 4 were significantly higher than those from the other sites during summer, autumn and winter 2001, while those from site 5 were significantly higher than those from the other sites during winter 2000 and spring ($p < 0.001$, n = 5 pool of 30 samples).

Table 53: Ni concentrations measured in soft tissue samples (µg/g ± SE, n = 5) of the mussel from the three sites during different seasons

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 4	5.34 ± 0.04	3.56 ± 0.04	9.76 ± 0.10	9.91 ± 0.10	10.20 ± 0.10
Site 5	9.17 ± 0.10	8.75 ± 0.10	0.20 ± 0.03	1.75 ± 0.03	2.35 ± 0.10
Site 6	2.04 ± 0.10	1.09 ± 0.10	ND	0.19 ± 0.10	1.17 ± 0.10

*ND- not detected (Site 4- Muizenberg; 5- Rooiels; 6- Kleinmond)

6.3.1.4. *Lead*

The mean Pb concentrations measured at site 5 were significantly higher ($p < 0.001$, n = 5 pool of 30 samples) than those from the other two sites during all the other seasons except winter 2001, when the site 4 concentrations became significantly higher than the others ($p < 0.001$) (Table 54). The mean Pb concentrations from site 6 (Appendix 39) were the lowest during all five seasons. One-way ANOVA indicated significant seasonal variations as well ($p < 0.001$).

Table 54: Pb concentrations measured in soft tissue samples (µg/g ± SE, n = 5) of the mussel at the three sites during the different seasons

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 4	11.56 ± 0.10	3.90 ± 0.11	9.76 ± 0.10	9.56 ± 0.10	15.56 ± 0.10
Site 5	15.63 ± 0.10	16.25 ± 0.10	10.08 ± 0.10	11.77 ± 0.10	12.05 ± 0.12
Site 6	0.63 ± 0.10	0.20 ± 0.10	0.07 ± 0.10	0.91 ± 0.10	1.17 ± 0.10

(Site 4- Muizenberg; 5- Rooiels; 6- Kleinmond)

6.3.1.5. *Zinc*

The mean Zn concentrations in the soft tissue samples obtained ranged between 52.44 (± 0.10) and 273.75 (± 0.10) µg/g dry weight (Table 55). The Zn values tended to decrease from winter 2000 to spring, and then increased again from autumn to winter 2001.

Table 55: Zn concentrations measured in the soft tissue samples (µg/g ± SE, n = 5) of the mussel at the three sites during different seasons

	Winter '00	Spring	Summer	Autumn '01	Winter '01
Site 4	69.22 ± 0.10	52.44 ± 0.10	58.45 ± 0.10	62.89 ± 0.10	179.05 ± 0.17
Site 5	273.75 ± 0.10	88.25 ± 0.10	152.90 ± 0.10	160.79 ± 0.11	185.0 ± 0.13
Site 6	121.88 ± 0.10	107.67 ± 0.12	90.67 ± 0.10	95.80 ± 0.10	101.70 ± 0.12

(Site 4- Muizenberg; 5- Rooiels; 6- Kleinmond)

One-way ANOVA indicated significant spatial differences in the mean Zn concentrations of the soft tissues ($p < 0.001$), with site 5 concentrations being significantly higher than those from the other sites during all the other seasons except spring, when site 6 concentrations became significantly higher ($p < 0.001$). There were also significant seasonal variations, with the winter 2000 values being significantly higher than those measured during the other seasons at sites 5 and 6 ($p < 0.001$), while the winter 2001 values were significantly higher than the others at site 4 ($p < 0.001$).

6.3.2. Heavy metal concentrations in the shell samples of *C. meridionalis*

6.3.2.1. *Cadmium*

The mean Cd concentrations in the shell samples ranged between 0.13 (± 0.10) and 7.75 (± 0.10) µg/g dry weight. The concentrations measured at the three sites tended to decrease progressively from winter to summer 2000 (Table 56). One-way ANOVA showed that these seasonal variations were highly significant ($p < 0.001$). There were also significant spatial variations in the shell mean concentrations ($p < 0.001$), with site 4 mean concentrations being significantly higher during winter 2000 and summer, while the site 5 mean concentrations were significantly higher during spring.

Table 56: Cd concentrations measured in shell samples (µg/g ± SE, n = 5) of the mussel from the different sites during different seasons

	Winter '00	Spring	Summer
Site 4	7.75 ± 0.10	2.78 ± 0.10	1.67 ± 0.10
Site 5	12.67 ± 0.10	6.11 ± 0.11	1.0 ± 0.10
Site 6	1.65 ± 0.10	0.33 ± 0.10	0.13 ± 0.10

(Site 4- Muizenberg; 5- Rooiels; 6- Kleinmond)

6.3.2.2. *Copper*

Table 57 shows the mean Cu concentrations obtained from the shell samples during the three seasons, from the three sampling sites. The site 4 mean Cu concentrations were significantly higher than those from the other sites during all three seasons sampled ($p < 0.001$). One-way ANOVA showed that there were significant seasonal variations in the shell mean Cu concentrations as well ($p < 0.001$).

TABLE 57: Mean Cu concentrations (µg/g ± SE, n = 5) measured in the shell samples of the mussel from the three sites during winter 2000, spring and summer (n = 5 pool of 30)

Seasons	**Site 4**	**Site 5**	**Site 6**
Winter 2000	2.67 ± 0.10	0.40 ± 0.10	ND
Spring 2000	2.22 ± 0.10	ND	ND
Summer 2000	0.83 ± 0.10	0.25 ± 0.10	ND

*ND- not detected (Site 4-Muizenberg; 5-Rooiels; 6-Kleinmond)

6.3.2.3. *Nickel*

The mean Ni concentrations measured in the shell samples from site 4 ranged between undetectable levels and 4.77 (± 0.12) µg/g dry weight, those from site 5 ranged between undetectable levels and 1.50 (± 0.10) µg/g, while no Ni was detected in the samples from site 6 (Table 58). One-way ANOVA indicated significant spatial differences in the shell mean Ni concentrations ($p < 0.001$), with site 5 concentrations

being significantly higher than those from the other sites during winter and spring, while site 4 concentrations were significantly higher than those from the other sites during summer. There were also significant seasonal variations in the Ni concentrations obtained at the different sites ($p < 0.001$). At site 4, the shell Ni concentrations decreased from winter 2000 to spring and then increased during summer. At site 5, the shell Ni concentrations decreased progressively from winter 2000, to undetectable levels during summer.

TABLE 58: Mean Ni concentrations (µg/g ± SE, n = 5) in the shell samples of the mussel from the three sites during winter, spring and summer 2000 (n = 5 pool of 30)

Seasons	Site 4	Site 5	Site 6
Winter 2000	2.67 ± 0.10	1.47 ± 0.11	8.33 ± 0.10
Spring 2000	4.77 ± 0.12	1.50 ± 0.10	ND
Summer 2000	ND	ND	ND

* ND- not detected (Site 4-Muizenberg; 5-Rooiels; 6-Kleinmond)

6.3.2.4. *Lead*

The mean Pb concentrations (Table 59) measured in the shell samples were in the order: site 4 > site 5 > site 6. The mean Pb concentrations ranged from undetectable levels, measured at site 6 during spring, to 25.75 (± 0.13) µg/g which was measured at site 4 during winter 2000. No Pb was detected in the shell samples from site 6 during spring and summer 2000. One-way ANOVA showed that these spatial variations in the Pb concentrations of shells were highly significantly ($p < 0.001$). There were also significant seasonal variations ($p < 0.001$) in the mean Pb concentrations in the shells, with the concentrations decreasing progressively from winter 2000 to summer, at all the sites.

TABLE 59: Mean Pb concentrations (µg/g ± SE, n = 5) measured in the shell samples of the mussel from the three sites during winter 2000, spring and summer (n = 5 pool of 30)

Seasons	Site 4	Site 5	Site 6
Winter 2000	25.75 ± 0.13	3.17 ± 0.14	0.20 ± 0.12
Spring 2000	5.70 ± 0.11	1.67 ± 0.10	ND
Summer 2000	6.83 ± 0.10	0.25 ± 0.13	ND

*ND- not detected (Site 4-Muizenberg; 5-Rooiels; 6-Kleinmond)

6.3.2.5. *Zinc*

The mean Zn concentrations measured in the shell samples from site 4 ranged between 37.95 (± 0.14) and 76.25 (± 0.11) µg/g dry weight (Table 60), at site 5 they ranged between 46.05 (± 0.13) and 93.75 (± 0.11) µg/g, while at site 6 they ranged between 43.75 (± 0.10) and 100.10 (± 0.14) µg/g. The Zn values measured at sites 5 and 6 were more or less similar during summer. The concentrations at sites 5 and 6 tended to decrease progressively from winter 2000 to summer. One-way ANOVA indicated significant seasonal and spatial differences in the mean Zn concentrations ($p < 0.001$, n = 5 pool of 30).

Table 60: Zn concentrations measured in the shell samples (µg/g ± SE, n = 5) of the mussel from the three sites during three different seasons

	Winter '00	Spring	Summer
Site 4	41.0 ± 0.14	37.95 ± 0.14	76.25 ± 0.11
Site 5	93.75 ± 0.11	81.75 ± 0.15	46.05 ± 0.13
Site 6	100.0 ± 0.14	73.04 ± 0.10	43.75 ± 0.10

(Site 4- Muizenberg; 5- Rooiels; 6- Kleinmond)

6.3.3. Comparison of heavy metal concentrations in the soft tissues and shells of *C. meridionalis*

6.3.3.1. *Cadmium*

The mean concentrations of the five heavy metals in the soft tissues and shells were compared for the three seasons (winter, spring and summer 2000) using t-tests. During winter and spring, the soft tissue mean Cd concentrations were significantly higher than the shell concentrations at site 5 ($p < 0.001$), while at site 4, the shell mean Cd concentrations were significantly higher than those of the soft tissues ($p < 0.001$) (Table 61). During summer, the soft tissue mean Cd concentrations were significantly higher than those of the shell samples at all the sites ($p < 0.001$)

TABLE 61: Comparison of Cd concentrations (µg/g ± SE, n = 5) measured during three seasons in soft tissues and shells of *C. meridionalis*

Site 4			
	Winter '00	Spring	Summer
Soft	2.78 ± 0.10	1.17 ± 0.10	8.09 ± 0.10
Shell	7.75 ± 0.10	2.78 ± 0.10	1.67 ± 0.10
Site 5			
Soft	16.25 ± 0.12	7.78 ± 0.10	3.0 ± 0.11
Shell	12.67 ± 0.10	6.11 ± 0.11	1.0 ± 0.10
Site 6			
Soft	3.69 ± 0.10	0.57 ± 0.10	0.67 ± 0.10
Shell	1.65 ± 0.10	0.33 ± 0.10	0.13 ± 0.10

(Site 4-Muizenberg; 5-Rooiels; 6-Kleinmond)

6.3.3.2. *Copper*

During winter 2000, the mean Cu concentrations measured in the soft tissues were significantly higher than those in the shell samples (Table 62) at all the sites ($p < 0.001$). During spring, the soft tissue mean concentrations were significantly higher than those of the shells at sites 5 and 6, while at site 4, the shell mean concentrations were significantly higher than those of the soft tissues ($p < 0.001$). During summer, the shell mean concentrations of Cu were significantly higher than those of the soft tissues at sites 4 and 6 ($p < 0.001$), while no Cu was detected in the samples from site 6 during the summer period.

TABLE 62: Comparison of Cu concentrations (µg/g ± SE, n = 5) measured during three seasons in soft tissues and shells of *C. meridionalis*

Site 4			
	Winter '00	Spring	Summer
Soft	3.44 ± 0.10	1.20 ± 0.12	0.36 ± 0.10
Shell	2.67 ± 0.10	2.22 ± 0.10	0.83 ± 0.10
Site 5			
Soft	5.0 ± 0.10	1.0 ± 0.10	ND
Shell	0.40 ± 0.10	ND	0.25 ± 0.10
Site 6			
Soft	3.69 ± 0.04	0.57 ± 0.04	ND
Shell	ND	ND	ND

*ND- not detected (Site 4-Muizenberg; 5-Rooiels; 6-Kleinmond)

6.3.3.3. *Nickel*

The soft tissue mean concentrations of Ni were significantly higher than those of the shells at all the sites during winter and spring 2000 ($p < 0.001$) (Table 63). During summer 2000, there were significantly higher Ni concentrations in the soft tissues

than in the shell samples at sites 4 and 5 ($p < 0.001$), while at site 6 no Ni was detected in both the soft tissues and shells.

TABLE 63: Comparison of Ni concentrations (µg/g ± SE, n = 5) measured during three seasons in soft tissues and shells of *C. meridionalis*

	Winter '00	Spring	Summer
Site 4			
Soft	5.34 ± 0.04	3.56 ± 0.04	9.76 ± 0.10
Shell	2.67 ± 0.10	4.77 ± 0.12	ND
Site 5			
Soft	9.17 ± 0.10	8.75 ± 0.10	0.20 ± 0.03
Shell	1.47 ± 0.11	1.50 ± 0.10	ND
Site 6			
Soft	2.04 ± 0.10	1.09 ± 0.10	ND
Shell	8.33 ± 0.10	ND	ND

*ND- not detected (Site 4-Muizenberg; 5-Rooiels; 6-Kleinmond)

6.3.3.4. *Lead*

The soft tissue mean concentrations of Pb were significantly higher ($p < 0.001$) than those of the shells during all three seasons at sites 5 and 6 (Table 64). At site 4, the soft tissue mean concentrations were significantly higher than those of the shell samples only during summer ($p < 0.001$), while the shell mean concentrations were significantly higher ($p < 0.001$) than those of the soft tissues during winter and spring 2009.

TABLE 64: Comparison of Pb concentrations (µg/g ± SE, n = 5) measured during three seasons in soft tissues and shells of *C. meridionalis*

Site 4			
	Winter '00	Spring	Summer
Soft	11.56 ± 0.10	3.90 ± 0.11	9.76 ± 0.10
Shell	25.75 ± 0.13	5.70 ± 0.11	6.83 ± 0.10
Site 5			
Soft	15.63 ± 0.10	16.25 ± 0.10	10.08 ± 0.10
Shell	3.17 ± 0.14	1.67 ± 0.10	0.25 ± 0.13
Site 6			
Soft	0.63 ± 0.10	0.20 ± 0.10	0.07 ± 0.10
Shell	0.20 ± 0.12	ND	ND

*ND- not detected (Site 4-Muizenberg; 5-Rooiels; 6-Kleinmond)

6.3.3.5. *Zinc*

At sites 5 and 6, the soft tissue mean concentrations of Zn were significantly higher ($p < 0.001$) than those of the shells during all three seasons (Table 65). At site 4, the soft tissue mean concentrations were significantly higher than those of the shells during winter and spring only ($p < 0.001$). During summer 2000, the shell mean concentrations at this site were significantly higher than those of the soft tissues ($p < 0.001$).

TABLE 65: Comparison of Zn concentrations (µg/g ± SE, n = 5) measured during three seasons in soft tissues and shells of *C. meridionalis*

Site 4			
	Winter '00	Spring	Summer
Soft	69.22 ± 0.10	52.44 ± 0.10	58.45 ± 0.10
Shell	41.0 ± 0.14	37.95 ± 0.14	76.25 ± 0.11
Site 5			
Soft	273.75 ± 0.10	88.25 ± 0.10	152.90 ± 0.10
Shell	93.75 ± 0.11	81.75 ± 0.15	46.05 ± 0.13
Site 6			
Soft	121.88 ± 0.10	107.67 ± 0.12	90.67 ± 0.10
Shell	100.0 ± 0.14	73.04 ± 0.10	43.75 ± 0.10

(Site 4-Muizenberg; 5-Rooiels; 6-Kleinmond)

6.3.4. Comparisons of all the heavy metals in the soft tissue and shell samples

In the soft tissue samples, the mean Zn concentrations were significantly higher ($p < 0.001$) than the other heavy metals at all the sites and during all five seasons sampled. In the shell samples, the mean Zn concentrations were significantly higher ($p < 0.001$) than the other heavy metals at all the sites during the three seasons sampled, that is, from winter to summer 2000.

6.4. DISCUSSION

The low mean salinity recorded in the present study in the water samples from site 5 during winter 2000 (Table 9, Chapter 2) may have contributed to the higher Cd concentrations in the water samples obtained from this site (Table 11, Chapter 2), resulting in the subsequent accumulation of significantly high levels of Cd in the soft tissues of *C. meridionalis* (Table 51). This is in agreement with previous findings elsewhere (Cunningham, 1979; Hops, 1990) that salinity has a strong influence on the

accumulation of metals by bivalves, and the view of various authors (Powell & White, 1990; Blackmore, 1999) that decreased salinity tends to increase the heavy metal uptake and accumulation by organisms. It is generally believed that the increase in metal accumulation with decreasing salinity is related to the increased free metal ion concentration as chloride complexation in the water decreases (Blackmore & Wang, 2002).

The accumulation of high Ni concentrations in the soft tissues of *C. meridionalis* at site 4 during winter 2001 (Table 53) may be related to the ambient levels determined earlier (Table 13, Chapter 2). The major repository organ for Ni in the mussels is, according to Eisler (1981), the digestive gland. The significantly higher levels of Ni in the ambient water at site 4 during winter 2001 compared to other seasons (Table 13, Chapter 2) may be associated with the sewage discharge from the numerous sewage outlets occurring at this site, especially during the rainy winter period (Taljaard et al. 2000), and, since Ni has a strong affinity for organic matter, may account for the higher Ni levels at this site.

Previous results (Watling & Watling, 1976) obtained for *C. meridionalis* at Bloubergstrand (1.51- 7.71 µg/g) and Saldanha Bay (3.00 µg/g dry weight) showed slightly lower levels of Ni than those in the present study. This could be an indication of higher pollution levels at the study site as a result of increased population pressure, development and industrial activity in recent years (Taljaard et al., 2000). The previous results obtained for *C. meridionalis* at Bloubergstrand and Saldanha Bay, however, indicated higher concentrations during summer (Watling & Watling, 1976). This may be related to the fact that the summer water temperatures along the west coast of the South African coastline, where the two areas are situated just north of the present study site (Figure 1, Chapter 2), are lower due to the cold upwelling of the Benguella current (Orren et al., 1980). These low summer temperatures found at the west coast correspond to the low winter temperatures normally found at the study site, which may explain the high metal concentrations in soft tissues being measured

during November in the other studies which correspond to the high winter values in the present study.

According to Phillips (1976), Cu is an essential element in mussels which forms part of the blood proteins In the present study, the Cu concentrations in the mussel soft tissues showed significant seasonal variations, which is in agreement with previous findings (Adler-Ivanbrook & Breslin, 1999). According to Kinne (1984), there is more Cu accumulation during the reproductive process. The mussels in the south-west coast of South Africa, which include those of the present study site, spawn during the September- October period (Watling & Watling (1976). The high Cu levels measured in the soft tissues during winter (Table 52) may thus be related to the gonad maturation process occurring prior to the spring spawning (Regoli, 1998). Another reason for the seasonal variation of Cu concentrations may be due to the rapid excretion of Cu by mussels compared to the other heavy metals such as Cd, Pb and Zn, as suggested by Nicholson (1999a) elsewhere. The Cu concentrations measured in the present study were found to be lower than those recorded previously for this species at Bloubergstrand (8.14 – 10.40 µg/g dry weight) and Saldanha Bay (14.00 µg/g) (Watling & Watling, 1976).

High levels of Pb are common in the soft tissues of shellfish collected near sewer outfalls, heavy traffic, industrialized or densely populated urban areas (Eisler, 1981). The significantly higher Pb concentrations in the soft tissues of mussels collected from site 5 (Table 54), and the shell samples from site 4 (Table 59) may, therefore, be the result of the urban stormwater runoff being discharged directly via the numerous stormwater outlets which open at site 4, and the Rooiels River mouth which opens at site 5 (DEAT, 1985). Another source of Pb in the mussels from this site may be related to the military weapons testing activities that took place in the catchment area of this site in the past (Cock & McKenzie, 1998), and which may have contributed to the accumulation of Pb in the sediments and mussel tissues over time. No sampling was undertaken in the former testing grounds to confirm this possibility.

Giamberini & Pihan (1997) and Yan et al. (1997) have suggested that mussels cannot regulate Pb, but instead accumulate it to high concentrations.

Calcareous skeletons have the ability to accumulate heavy metals to several orders of magnitude above those found in the environment (Chinchon et al., 2000), which may explain the presence of heavy metals in the shell compartment of the mussel body (Tables 61-65). According to Kinne (1984), another route for the removal or detoxification of Pb is by incorporating it into shells, which may explain the presence of significantly high levels of Pb in the shell samples from site 4 (Table 59). According to Sauer & Watabe (1989), Pb appears to be metabolized via the Ca pathways and accumulated into the shell tissues. The results of the present study (Table 54) indicate that the Pb concentrations measured in this species were almost double the levels previously measured at Bloubergstrand (1.19 – 4.16 µg/g) and at Saldanha Bay (5.00 µg/g) (Watling & Watling, 1976).

Previous concentrations obtained for Zn in this species at Bloubergstrand (88.20 – 118.00 µg/g) and at Saldanha Bay (113.00 µg/g) (Watling & Watling, 1976) were slightly lower than those measured in the present study (Table 55). It has been suggested that the greatest concentration of Zn in marine biota is found in filter-feeding molluscs (Eisler, 1981), which is in agreement with the present results where the mean Zn concentrations were significantly higher than the other heavy metals, in both the soft tissue and shell compartments of the body. The source of Zn may be domestic and industrial effluent (Taljaard et al., 2000), although, according to Moore (1981), the major source of Zn uptake and accumulation in molluscs is probably the food rather than uptake from the seawater. According to Frias-Espericueta et al. (1999), Zn is the key element regarding the reproductive cycle in mussels, and may be transferred from the somatic to the gonadal tissue.

Generally, the mussels collected from sites 4 and 5 had higher heavy metal concentrations in their soft tissues than those from site 6 (Tables 51 - 55). This may be related to the enclosed nature of False Bay, which results in longer residence time of

pollutants and accumulation of contaminants (Taljaard et al., 2000), compared to the exposed coastal area where site 6 is located. There may thus be higher flushing rates at site 6, leading to the lower concentrations of heavy metals being accumulated by the animals collected from this site, compared to those from the two other sites which are situated within the bay.

The significantly higher metal concentrations measured in the water and soft tissues during the two winter periods in the present study may be related to the increased runoff rate resulting from the increased precipitation during the rainy winter period (Table 10, Chapter 2) (Taljaard et al., 2000). The increased runoff may have resulted in the mobilization of heavy metals from diffuse sources into the storm-water drains that eventually discharge into False Bay (Taljaard et al., 2000).

6.5. CONCLUSION

The mussel *C. meridionalis* showed seasonal variations in the heavy metal concentrations. The seasonal changes observed in the metal content may be related to gonad development and spawning, as well as to the changing environmental conditions. The results of the present study have confirmed the fact that shells can act as receptors of pollutants, and that the shell metal levels may be an indication of the environmental presence of heavy metals. The species showed higher concentrations of most heavy metals compared to previous studies carried out in South Africa, which may be an indication of increased contamination levels at present compared to the situation in the past. The soft tissue and shell concentrations of heavy metals may be an indication of the accumulation of heavy metals from the water medium by this filter-feeding organism, thus making it a suitable biomonitor of heavy metal contamination in the study area.

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CHAPTER 7- HEAVY METAL CONCENTRATIONS IN THE SEA STAR *PATIRIELLA EXIGUA* (ECHINODERMATA: ASTEROIDEA) FROM FALSE BAY

7.1. INTRODUCTION

Investigations into the interactions between trace metals and marine organisms became intensified because of increased anthropogenic inputs of these metals into aquatic systems (Engel et al., 1981). The Minamata incident which occurred in Japan in the 1950s when people were poisoned with mercury after consuming poisoned shellfish (Itakura et al., 1999) brought home the problem of marine contamination from waste disposal. Following that incident, heavy metals in the oceans became of widespread concern, with research directed at the distribution and fate of heavy metals in the marine environment (McIntyre, 1995).

The majority of contaminants entering the marine environment from land-based sources tends to be delivered to the near-shore environments, where they become trapped and cycled (Windom, 1992). There is a need, therefore, to develop benthic assays using ecologically important organisms that are relevant to the habitat in which potential pollution is occurring (Newton & McKenzie, 1995). There is a lack information on the heavy metal metabolism and sublethal effects of heavy metals on echinoderms, although the results of previous studies found these organisms to be valuable bioindicators of spatial and temporal Cd and Pb contamination in the field (Eisler, 1981).

Echinoderms are cosmopolitan in nature, with almost all being bottom-dwellers (Eisler, 1981), and inhabiting a variety of subtidal substrata ranging from coarse gravel, to rocks and fine mud (Freeman et al., 2001). The response of echinoderms to pollutants is of ecological relevance since they occur in most marine habitats and are the abundant and dominant species in many communities (Newton & McKenzie, 1995), hence their inclusion in the present study. The aim of this part of the study was to determine the concentrations of the five heavy metals (Cd, Cu, Ni, Pb and Zn) in the whole-body samples of the sea star species *P. exigua,* commonly found in the

False Bay intertidal zone, and to compare these to the levels in the water and sediments.

7.2. MATERIALS AND METHODS

7.2.1. Sampling and heavy metal extraction and analysis

Specimens of *P. exigua* were hand-collected from beneath the rocks found in the shallow pools of five sites where the species occurs abundantly (sites 1, 2, 3, 4 and 7) during low-tide, over five seasons. None were found at the other two sites of the study area. Fifteen specimens (0.2-1.0 cm diameter) were collected from each site and put in plastic buckets containing site water for transporting to the laboratory. Water and sediment samples were collected simultaneously from the same sites, put in plastic buckets which were then placed in cooler boxes containing ice for transporting to the laboratory. The water temperature was measured on site with a thermometer, while the pH and salinity were measured at the laboratory with the pH and salinometer respectively. At the laboratory, the animals were weighed, oven-dried for 48 hours at 60°C, and then the pooled samples from each site were homogenized to a powder using a pestle and mortar. Five replicate aliquots (0.2-0.3 g) were placed in acid-cleaned test tubes and acid-digested with nitric and perchloric acids as described in previous chapters (p. 18). Heavy metal concentrations were determined using the AAS method, and expressed as µg/g dry weight.

7.2.2. Statistical analysis

One-way ANOVA was used to determine the seasonal and spatial variations in the whole-body concentrations of *P. exigua*. The heavy metal concentrations of the body, samples were also compared to those of the water and sediments measured at the sites using one-way ANOVA. The Pearson correlation analyses were used to determine the relationship between the water parameters and whole-body concentrations, as well as those between the body, water and sediment concentrations.

7.3. RESULTS

7.3.1. Water parameters

7.3.1.1. Water salinity

The lowest salinity values measured during all five seasons were recorded at site 1 (Table 9, Chapter 2). One-way ANOVA showed that while significant seasonal differences in salinity occurred ($p < 0.001$), there were, however, no significant spatial differences ($p > 0.05$).

7.3.1.2. Water temperatures

Table 66 shows the mean water temperatures measured at the sites during the different seasons. There was a rapid decrease in the mean temperatures from summer to autumn, with the means measured at site 7 being slightly lower than those of the other sites. One-way ANOVA showed that these seasonal differences in the mean temperatures were highly significant ($p < 0.001$).

TABLE 66: Mean water temperatures (°C ± SE, n = 5) measured at the different sites during five seasons, N = 3

Sites	Winter '00	Spring	Summer	Autumn	Winter '01
1	14.00 ± 0.12	18.02 ± 0.10	24.11 ± 0.10	17.16 ± 0.10	15.00 ± 0.11
2	14.10 ± 0.13	18.10 ± 0.12	25.05 ± 0.121	18.00 ± 0.10	15.12 ± 0.12
3	14.40 ± 0.10	17.05 ± 0.12	19.10 ± 0.11	15.17 ± 0.10	14.20 ± 0.10
4	14.12 ± 0.10	18.11 ± 0.10	19.20 ± 0.12	15.05 ± 0.10	14.70 ± 0.10
5	14.80 ± 0.12	18.15 ± 0.12	23.03 ± 0.10	14.09 ± 0.10	14.90 ± 0.12
6	14.10 ± 0.04	18.17 ± 0.10	22.06 ± 0.04	16.10 ± 0.04	16.00 ± 0.10
7	13.30 ± 0.10	16.01 ± 0.11	18.40 ± 0.10	14.14 ± 0.10	15.10 ± 0.12

7.3.1.3. Water pH

The water pH (Table 67) ranged between 7.10 (± 0.10) and 8.67 (± 0.10). During winter 2000, the pH ranged between 7.10 (± 0.10) and 7.53 (± 0.10). The spring and summer values increased slightly, and ranged between 7.15 (± 0.12) and 8.67 (± 0.10). The mean pH values decreased again during autumn and winter 2001, and ranged between 7.22 (± 0.10) and 7.80 (± 0.10).

One-way ANOVA showed that the spring and summer values were significantly higher than those measured during winter and autumn ($p < 0.001$).

TABLE 67: Mean water pH (± SE, n = 5) measured at the different sites during five seasons, N = 3

Sites	Winter '00	Spring	Summer	Autumn	Winter '01
1	7.20 ± 0.12	8.06 ± 0.10	8.17 ± 0.10	7.49 ± 0.10	7.30 ± 0.10
2	7.15 ± 0.11	8.14 ± 0.10	8.23 ± 0.110	7.77 ± 0.10	7.45 ± 0.12
3	7.53 ± 0.10	8.12 ± 0.10	8.27 ± 0.12	7.36 ± 0.10	7.10 ± 0.12
4	7.20 ± 0.11	7.93 ± 0.10	8.67 ± 0.10	7.29 ± 0.12	7.22 ± 0.10
5	7.10 ± 0.10	8.32 ± 0.10	8.20 ± 0.10	7.41 ± 0.10	7.30 ± 0.10
6	7.20 ± 0.10	7.15 ± 0.12	7.35 ± 0.10	7.60 ± 0.10	7.80 ± 0.10
7	7.30 ± 0.11	8.01 ± 0.10	8.31 ± 0.10	7.30 ± 0.10	7.40 ± 0.10

7.3.2. Heavy metal concentrations

7.3.2.1. *Cadmium*

Table 68 shows the mean Cd concentrations measured in the water, sediment and whole-body samples during winter 2000. The water concentrations ranged between undetectable levels at site 7, and 0.75 (± 0.03) µg/L, which was measured at site 4. The sediment concentrations ranged between 0.20 (± 01) at site 7, and 12.36 (± 0.02) µg/g, which was measured at site 4. The whole-body concentrations ranged between 3.33 (± 0.10) at site 2, and 9.00 (± 0.12) µg/g, which was measured at site 7. The mean sediment concentrations of Cd at site 4 (12.36 µg/g) were significantly higher than those of the water (0.15 µg/g) and body (7.25 µg/g) samples ($p < 0.001$). At the other sites (2, 3 and 7) the mean body concentrations were significantly higher than those of water and sediments ($p < 0.001 n = 5$).

Table 68: The Cd concentrations measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during winter 2000, n = 5

	Water	Sediment	Body
Site 1	0.15 ± 0.02	7.36 ± 0.21	5.25 ± 0.12
Site 2	0.65 ± 0.03	3.03 ± 0.10	3.33 ± 0.10
Site 3	0.05 ± 0.01	0.21 ± 0.02	4.75 ± 0.12
Site 4	0.75 ± 0.04	12.36 ± 0.21	7.25 ± 0.11
Site 7	ND	0.20 ± 0.01	9.0 ± 0.12

*ND- not detected; (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During spring 2000, the mean water concentrations ranged between undetectable levels at site 7, and 0.55 (± 0.02) µg/L, which was measured in the samples from site 4 (Table 69). The mean sediment concentrations ranged between 0.10 (± 0.02), which was measured at site 3, and 2.77 (± 0.02) µg/g, which was measured at site 4. The mean body concentrations ranged between 0.05 (± 0.02) at site 7, and 5.07 (± 0.11) µg/g at site 4. One-way ANOVA showed that the mean sediment concentrations were significantly higher than those of the water and body samples at sites 2 and 7 ($p < 0.001$). At sites 1, 3 and 4, the body concentrations became significantly higher than those of the water and sediments ($p < 0.001$).

Table 69: The Cd concentrations measured in the water (µg/L), sediment and body samples (µg/g) of *P exigua* from the different sites during spring 2000, n = 5

	Water	Sediment	Body
Site 1	0.20 ± 0.02	1.54 ± 0.02	2.15 ± 0.10
Site 2	0.04 ± 0.01	1.01 ± 0.01	0.34 ± 0.10
Site 3	0.15 ± 0.02	0.10 ± 0.02	3.0 ± 0.11
Site 4	0.55 ± 0.03	2.77 ± 0.03	5.07 ± 0.11
Site 7	ND	0.30 ± 0.01	0.05 ± 0.02

*ND- not detected; (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During summer 2000, the mean water concentrations of Cd measured in site 3 samples were significantly higher than those of the water samples from the other sites ($p < 0.001$) (Table 70). The sediment concentrations of samples obtained from site 4 were significantly higher than those of the sediments from the other sites ($p < 0.001$). The body concentrations of samples from site 1 (6.52 ± 0.10 µg/g) were significantly higher than those of the body samples obtained from the other sites ($p < 0.001$).

Table 70: The Cd concentrations measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during summer 2000, n = 5

	Water	Sediment	Body
Site 1	1.05 ± 0.03	3.0 ± 0.04	6.52 ± 0.10
Site 2	1.0 ± 0.02	5.25 ± 0.20	1.52 ± 0.10
Site 3	3.24 ± 0.10	ND	2.28 ± 0.10
Site 4	1.37± 0.10	10.75 ± 0.02	2.0 ± 0.10
Site 7	0.15 ± 0.02	1.30 ± 0.01	3.26 ± 0.10

*ND- not detected; (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During autumn 2001, the mean water concentrations of Cd ranged between 1.49 (± 0.01) and 6.60 (± 0.02) µg/L, which were measured in samples obtained from site 7 and 3 respectively (Table 71). One-way ANOVA showed that site 3 concentrations were significantly higher than those from the other sites ($p < 0.001$).The mean sediment concentrations ranged between 0.21 (± 0.01) and 10.46 (± 0.01) µg/g, which were measured in samples from sites 3 and 4 respectively. One-way ANOVA showed that the mean sediment concentration of site 4 samples was significantly higher ($p < 0.001$) than those of the samples obtained from the other sites. The mean body concentrations of Cd ranged between 3.04 (± 0.11) and 5.25 (± 0.02) µg/g, which were measured at sites 3 and 1 respectively.

Table 71: The Cd concentrations measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during autumn 2001, n = 5

	Water	Sediment	Body
Site 1	2.0 ± 0.10	3.09 ± 0.01	5.25 ± 0.10
Site 2	1.94 ± 0.04	6.17± 0.01	4.33 ± 0.10
Site 3	6.60 ± 0.10	0.21 ± 0.01	3.04 ± 0.11
Site 4	2.01± 0.02	10.46 ± 0.01	3.97 ± 0.13
Site 7	1.49 ± 0.02	1.59 ± 0.01	3.84 ± 0.10

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

The mean Cd concentrations measured in the various samples during winter 2001 are shown in Table 72. The water concentrations measured at sites 1, 2 and 4 were more or less similar. The mean water concentration of samples obtained from site 3 was significantly higher than those of the samples obtained from the other sites ($p < 0.001$). The mean concentrations of the sediment samples ranged between 1.21 (± 0.02) and 11.79 (± 0.01) µg/g, which were measured at sites 3 and 4 respectively. One-way ANOVA showed that the latter was significantly higher than those from the other sites ($p < 0.001$). The mean body concentrations ranged between 4.00 (± 0.12) and 11.50 (± 0.11) µg/g, with the latter mean concentration being measured in the samples from site 1 being significantly higher than those from the other sites ($p < 0.001$). The mean concentrations of the body samples obtained from sites 3 and 7 were more or less similar (4.00 ± 0.12).

Table 72: The Cd concentrations measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during winter 2001, n = 5

	Water	Sediment	Body
Site 1	2.49 ± 0.10	5.16 ± 0.25	11.50 ± 0.11
Site 2	2.47 ± 0.10	6.90 ± 0.01	7.50 ± 0.10
Site 3	6.94 ± 0.04	1.21 ± 0.22	4.0 ± 0.12
Site 4	2.40 ± 0.02	11.79 ± 0.28	5.25 ± 0.12
Site 7	1.88 ± 0.02	1.67 ± 0.02	4.17 ± 0.10

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

7.3.2.2. *Copper*

During winter 2000, the mean water concentrations of Cu ranged between undetectable levels and 2.20 (± 0.02) µg/L, and were in the order: site 3 > site 2 > site 4, with none being detected in the water samples from sites 1 and 7 (Table 73). The sediment concentrations ranged between 3.18 (± 0.01) and 15.10 (± 0.03) µg/g, and were in the order: site 1 > site 7 > site 4 > site 2 > site 3. One-way ANOVA indicated significant spatial differences in the mean sediment concentrations of Cu ($p < 0.001$). The body concentrations ranged between 3.48 (± 0.11) and 41.75 (± 0.11) µg/g, and were in the order: site 4 > site 3 > site 1 > site 2 > site 7. One-way ANOVA indicated significant spatial differences in the mean body concentrations ($p < 0.001$). One-way ANOVA also showed that the mean body concentrations were significantly higher than those of the water and sediment samples ($p < 0.001$) at all other sites except at site 7, where the mean sediment concentrations were significantly higher than those of the water and body samples ($p < 0.001$).

Table 73: The Cu concentrations measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during winter 2000, n = 5

	Water	Sediment	Body
Site 1	ND	15.10 ± 0.01	26.25 ± 0.10
Site 2	1.55 ± 0.02	3.18 ± 0.02	16.50 ± 0.10
Site 3	2.20 ± 0.02	1.16 ± 0.01	30.25 ± 0.16
Site 4	0.60 ± 0.04	5.27 ± 0.01	41.75 ± 0.11
Site 7	ND	11.0 ± 0.03	3.48 ± 0.10

*ND- note detected; (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During spring 2000, no Cu was detected in the water samples from all the other sites except those from site 4 (0.60 ± 0.03 µg/g). In the sediments, Cu was only detected in those samples which were obtained from site 4 (1.41 ± 0.02 µg/g) and site 7 (2.71 ± 0.01 µg/g). The body concentrations ranged between 2.05 (± 0.10) and 23.00 (± 0.01) µg/g, with the highest being measured at site 4. The body concentrations measured at sites 1 and 2 were similar (12.36 ± 0.10µg/g). One-way ANOVA indicated significant spatial differences in the mean body concentrations of Cu during spring ($p < 0.001$).

The Cu concentrations, which were measured in the different samples during summer 2000 are shown in Table 74. The mean concentrations obtained in the water samples from sites 1 and 2 were similar, while no Cu was detected in the water samples from site 7. In the sediments, Cu was detected only at site 7. The mean body concentrations ranged between 3.27 (± 0.10) and 6.65 (± 0.02) µg/g, which were measured at sites 2 and 1 respectively. One-way ANOVA indicated significant spatial differences in the mean body concentrations of Cu. The mean body concentrations were also significantly higher than those of the water and sediment samples at all sites ($p < 0.001$).

TABLE 74: The mean concentrations of Cu measured in the water (µg/L), sediments and body samples (µg/g) of *P. exigua* during summer, n = 5

	Water	Sediment	Body
Site 1	0.67 ± 0.02	ND	6.65 ± 0.12
Site 2	0.67 ± 0.02	ND	3.27 ± 0.10
Site 3	0.12 ± 0.02	ND	5.0 ± 0.10
Site 4	0.4 ± 0.03	ND	4.55 ± 0.10
Site 7	ND	1.94 ± 0.01	3.92 ± 0.10

*ND- not detected; (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During autumn 2001, the water concentrations of Cu ranged between 0.95 (± 0.02) and 2.30 (± 0.01) µg/L, with the latter mean concentration, which was measured at site 7, being slightly higher than those from the other sites (Table 75). In the sediment samples, the mean Cu concentrations ranged between 0.98 (± 0.01) and 11.50 (± 0.02) µg/g. The mean body concentrations ranged between 3.50 (± 0.11) and 11.70 (± 0.010.10) µg/g. One-way ANOVA indicated significant spatial differences in the mean sediment and body Cu concentrations ($p < 0.001$).

TABLE 75: The mean concentrations of Cu measured in the water (µg/L), sediments and body samples (µg/g) of *P. exigua* during autumn 2001, n = 5

	Water	Sediment	Body
Site 1	1.10 ± 0.03	1.24 ± 0.01	11.70 ± 0.10
Site 2	0.95 ± 0.04	0.98 ± 0.01	3.50 ± 0.11
Site 3	1.84 ± 0.03	6.05 ± 0.02	5.25 ± 0.10
Site 4	1.22 ± 0.01	7.46 ± 0.01	6.14 ± 0.10
Site 7	2.30 ± 0.02	11.50 ± 0.02	4.17 ± 0.10

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During winter 2001, the mean water concentrations of Cu ranged between 1.15 (± 0.02) and 3.10 (± 0.02) µg/L, and were in the order: site 4 > site 7 > site 3 > site 1 > site 2 (Table 76). The mean sediment concentrations were in the order site 7 > site 4 > site 2 > site 1 > site 3. One-way ANOVA showed that site 7 mean sediment Cu concentration was significantly higher than those from the other sites ($p < 0.001$), while the mean body concentration from site 3 was significantly higher ($p < 0.001$) than those from the other sites.

TABLE 76: The mean concentrations of Cu measured in the water (µg/L), sediments and body samples (µg/g) of *P. exigua* during winter 2001, n = 5

	Water	Sediment	Body
Site 1	2.09 ± 0.10	6.77± 0.01	16.50± 0.11
Site 2	1.15 ± 0.02	6.96 ± 0.02	11.0 ± 0.11
Site 3	2.30± 0.03	6.10 ± 0.01	17.97 ± 0.10
Site 4	3.10 ± 0.02	8.46 ± 0.01	9.15 ± 0.15
Site 7	2.45 ± 0.03	12.0 ± 0.10	5.25 ± 0.13

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

7.3.2.3. *Nickel*

During winter 2000, Ni was not detected in the water samples from sites 1, 2 and 7 (Table 77), while mean concentrations ranging between 0.15 (± 0.02) and 1.52 (± 0.10) µg/L were measured at sites 4 and 3 respectively. In the sediments, the samples from site 7 had a significantly higher mean concentration of Ni ($p < 0.001$) compared to the other sites. The mean body concentrations ranged between 0.01 (± 0.01) and 2.25 (± 0.12) µg/g, with the mean concentration measured at site 1 being significantly higher than those from the other sites ($p < 0.001$).

TABLE 77: The mean concentrations of Ni measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during winter 2000, n = 5

	Water	Sediment	Body
Site 1	ND	12.21 ± 0.03	2.25 ± 0.12
Site 2	ND	24.24 ± 0.27	0.05 ± 0.03
Site 3	1.52 ± 0.10	6.42 ± 0.17	0.51 ± 0.10
Site 4	0.15 ± 0.02	18.18 ± 0.10	0.01 ± 0.01
Site 7	ND	29.5 ± 0.15	0.02 ± 0.01

* ND- not detected; Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point

During spring 2000, Ni was detected only in the water samples from site 4 (Table 78). In the sediments, the mean Ni concentrations ranged between 0.52 (± 0.02) and 17.50 (± 0.01) µg/g. One-way ANOVA indicated significant spatial differences in the mean Ni concentrations of the sediments ($p < 0.001$). In the body samples, Ni was detected only in those samples which were obtained from site 1.

TABLE 78: The mean Ni concentrations measured in the water (µg/L), sediments and body samples (µg/g) of *P. exigua* from the different sites during spring 2000, n = 5

	Water	Sediment	Body
Site 1	ND	17.5 ± 0.03	1.0 ± 0.11
Site 2	ND	16.9 ± 0.31	ND
Site 3	ND	0.52 ± 0.02	ND
Site 4	0.5 ± 0.02	9.4 ± 0.21	ND
Site 7	ND	15.79 ± 0.20	ND

*ND- not detected; Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point

Table 79 shows the mean Ni concentrations measured in the water, sediment and body samples during summer 2000. The mean water concentrations ranged between undetectable levels and 5.20 (± 0.03) µg/L, the mean sediment concentrations between 9.63 (± 0.10) and 50.00 (± 0.01) µg/g, while Ni was not detected in any of the body samples. One-way ANOVA indicated significant spatial differences in the sediment concentrations ($p < 0.001$).

TABLE 79: The mean Ni concentrations measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during summer 2000, n = 5

	Water	Sediment	Body
Site 1	ND	22.5 ± 0.25	ND
Site 2	2.2 ± 0.02	30.25 ± 0.12	ND
Site 3	5.2 ± 0.10	13.04 ± 0.24	ND
Site 4	5.08 ± 0.03	50.0 ± 0.38	ND
Site 7	ND	9.63 ± 0.10	ND

*ND- not detected; Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point

During autumn 2001, the mean water concentrations of Ni ranged between 0.15 and 6.49 (0.02) µg/L (Table 80), with the highest being measured at site 3. The mean sediment concentrations ranged between 11.59 (± 0.20) and 37.27(± 0.16) µg/g, with those measured at site 2 being significantly higher than those measured from the other sites ($p < 0.001$). The mean body concentrations ranged between 0.50 (± 0.10) and 1.77 (± 0.10) µg/g, which were measured at sites 2 and 3 respectively.

TABLE 80: The mean Ni concentrations measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during autumn 2001, n = 5

	Water	Sediment	Body
Site 1	1.00 ± 0.04	30.29± 0.33	1.50± 0.10
Site 2	3.45 ± 0.02	37.27 ± 0.16	0.50 ± 0.10
Site 3	6.48 ± 0.03	16.71 ± 0.26	1.77 ± 0.10
Site 4	6.30 ± 0.02	30.86 ± 0.02	1.44 ± 0.10
Site 7	0.15 ± 0.02	11.59 ± 0.20	0.97 ± 0.13

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During winter 2001, the mean water concentrations of Ni ranged between 1.30 (± 0.03) and 10.74 (± 0.02) µg/L, which were measured at sites 7 and 4 respectively (Table 81). In the sediments, the mean Ni concentrations ranged between 19.91 (± 0.13) and 46.82 (± 0.01) µg/g, which were measured at sites 3 and 4 respectively. One-way ANOVA indicated significant spatial differences in the mean water and sediment Ni concentrations ($p < 0.001$). The mean body concentrations ranged between 1.00 (± 0.12) and 6.00 (± 0.02) µg/g, with the mean concentration measured from site 4 samples being significantly higher ($p < 0.001$) than those from the other sites. One-way ANOVA also showed that the sediment concentrations were always significantly higher than those of the water and body samples in all the sites and during all five seasons ($p < 0.001$).

TABLE 81: The mean Ni concentrations measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during winter 2001, n = 5

	Water	Sediment	Body
Site 1	2.06 ± 0.02	32.99± 0.44	4.50 ± 0.11
Site 2	6.35 ± 0.04	38.01 ± 0.18	1.00 ± 0.12
Site 3	8.30 ± 0.02	19.91 ± 0.13	5.75 ± 0.14
Site 4	10.74 ± 0.02	46.82 ± 0.01	6.00 ± 0.10
Site 7	1.30 ± 0.03	31.50 ± 0.03	1.50 ± 0.13

7.3.2.4. *Lead*

Table 82 shows the mean Pb concentrations measured in the water, sediments and body samples during winter 2000. The mean water concentrations were in the order: site 3 > site 7 > site 2 > site 4 > site 1. The mean sediment concentrations were in the order: site 2 > site 7 > site 4 > site 1 > site 3. One-way ANOVA showed that these spatial variations were highly significant ($p < 0.001$). The mean body concentrations were in the order: site 4 > site 3 > site 2 > site 1 > site 7, with significant spatial differences found in these values ($p < 0.001$).

TABLE 82: The mean concentrations of Pb measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during winter 2000, n = 5

	Water	Sediment	Body
Site 1	0.14 ± 0.03	15.15 ± 0.10	2.35 ± 0.11
Site 2	0.5 ± 0.04	60.76 ± 0.10	5.0 ± 0.10
Site 3	1.15 ± 0.02	10.84 ± 0.10	11.75 ± 0.11
Site 4	0.3 ± 0.02	28.41 ± 0.02	15.75 ± 0.10
Site 7	0.67 ± 0.01	41.0 ± 0.04	1.0 ± 0.14

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During spring 2000, the values of the mean Pb concentrations in the water samples ranged between 0.04 (± 0.02) and 10.17 (± 0.02) µg/L, with the mean concentration measured in the water samples from site 4 being significantly higher than those from the other sites ($p < 0.001$) (Table 83). The mean sediment concentrations ranged between 2.00 (± 0.02) and 47.28 (± 0.02) µg/g, with the mean concentration from site 4 being significantly higher than those from the other sites ($p < 0.001$). The mean Pb concentration of the body samples ranged between 0.80 (± 0.10) µg/g, which was measured at site 7, and 4.00 (± 0.01) µg/g, which was measured at site 4. One-way ANOVA showed that these spatial variations were highly significant ($p < 0.001$).

TABLE 83: The mean concentrations of Pb measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during spring 2000, n = 5

	Water	Sediment	Body
Site 1	0.15 ± 0.02	2.0 ± 0.02	2.0 ± 0.15
Site 2	0.35 ± 0.01	38.46 ± 0.04	2.33 ± 0.10
Site 3	0.35 ± 0.02	3.68 ± 0.04	0.13 ± 0.10
Site 4	10.17 ± 0.04	47.82 ± 0.10	4.0 ± 0.13
Site 7	0.04 ± 0.02	22.90 ± 0.10	0.80 ± 0.12

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During summer 2000, the mean water concentrations ranged between 0.35 (± 0.02) and 5.20 (± 0.03) µg/L, which were measured at sites 7 and 4 respectively (Table 84). The mean sediment concentrations measured at the various sites differed significantly, with those from site 2 being significantly higher ($p < 0.001$) than those from the other sites. In the body samples, the values ranged between 0.23 (± 0.10) and 2.73 (± 0.11) µg/g, with the lowest mean concentrations being measured in the samples obtained from both sites 1 and 2.

TABLE 84: The mean concentrations of Pb measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during summer 2000, n = 5

	Water	Sediment	Body
Site 1	1.70 ± 0.02	10.08 ± 0.03	0.24 ± 0.10
Site 2	1.05 ± 0.02	35.08 ± 0.04	0.23 ± 0.10
Site 3	4.80 ± 0.02	30.08 ± 0.02	2.0 ± 0.11
Site 4	5.20 ± 0.03	28.64 ± 0.10	2.73 ± 0.10
Site 7	0.35 ± 0.02	23.33 ± 0.02	1.09 ± 0.10

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

The mean Pb concentrations measured in the water samples during autumn 2001 ranged between 2.20 (± 0.02) and 6.30 (± 0.02) µg/L (Table 85). The mean sediment concentrations ranged between 16.80 (± 0.10) and 37.73 (± 0.10) µg/g, with significant spatial differences being found ($p < 0.001$). The mean body concentrations ranged between 1.33 (± 0.10) and 3.19 (± 0.10) µg/g, with the lowest mean concentration being measured at site 2, and the highest at site 4.

TABLE 85: The mean concentrations of Pb measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during autumn 2001, n = 5

	Water	Sediment	Body
Site 1	2.20 ± 0.02	16.80 ± 0.10	1.50 ± 0.11
Site 2	2.84 ± 0.02	36.06 ± 0.02	1.33 ± 0.10
Site 3	6.30 ± 0.02	34.65 ± 0.02	2.79 ± 0.10
Site 4	4.30 ± 0.10	37.73 ± 0.10	3.19 ± 0.10
Site 7	3.40 ± 0.03	30.70 ± 0.04	2.78 ± 0.12

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

Table 86 shows the mean Pb concentrations measured in the various samples during winter 2001. The mean water concentrations ranged between 5.20 (± 0.03) and 14.00 (± 0.10) µg/L, with those from site 1 being significantly higher than the others ($p < 0.001$). The mean sediment concentration of Pb ranged between 17.00 (± 0.10) and 41.50 (± 0.14) µg/g, with the samples obtained from site 4 having a significantly higher mean concentration than those from the other sites ($p < 0.001$). The mean body concentrations ranged between 2.00 (± 0.13) and 5.00 (± 01) µg/g, which were measured at sites 1 and 4 respectively. One-way ANOVA showed that all the sediment samples had significantly higher mean Pb concentrations than those of the water and body samples ($p < 0.001$). This was the case during all seasons and at all the other sites except at site 3, when the body concentration of Pb was higher than those of the water and sediments during winter 2000.

TABLE 86: The mean concentrations of Pb measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during winter 2001, n = 5

	Water	Sediment	Body
Site 1	14.0 ± 0.10	17.0 ± 0.10	2.0 ± 0.13
Site 2	6.30 ± 0.02	40.49 ± 0.16	4.73 ± 0.10
Site 3	9.20 ± 0.01	34.74 ± 0.02	3.45 ± 0.14
Site 4	11.40 ± 0.02	41.50 ± 0.14	5.0 ± 0.12
Site 7	5.20 ± 0.03	41.50 ± 0.02	3.60 ± 0.11

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

7.3.2.5. *Zinc*

Table 87 shows the mean Zn concentrations which were measured during winter 2000. In the water samples, significant spatial differences were found, with those from site 4 being significantly higher ($p < 0.001$) than those from the other sites. The values of the mean water concentrations ranged between 3.35 (± 0.02) and 30.15 (± 0.03) µg/L, and were in the order: site 4 > site 3 > site 2 > site 7 > site 1. The values in the sediment samples ranged between 26.73 (± 0.10) and 72.71 (± 0.03) µg/ml, and were

in the order: site 2 > site 7 > site 1 > site 4 > site 3. The mean body samples ranged between 35.00 and 224.75 (± 0.02) µg/g, which were measured at sites 7 and 1 respectively. One-way ANOVA indicated significant spatial differences in the mean Zn concentrations of the sediment and body samples ($p < 0.001$).

TABLE 87: The mean concentrations of Zn measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during winter 2000, n = 5

	Water	Sediment	Body
Site 1	3.35 ± 0.02	38.24 ± 0.02	224.75 ± 0.11
Site 2	16.95 ± 0.02	72.71 ± 0.03	76.0 ± 0.14
Site 3	24.60 ± 0.03	14.10 ± 0.04	151.75 ± 0.10
Site 4	30.15 ± 0.03	26.73 ± 0.10	149.25 ± 0.12
Site 7	4.50 ± 0.02	67.50 ± 0.03	35.0 ± 0.10

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During spring, the water concentrations ranged between 1.90 (± 0.02) and 12.30 (± 0.03) µg/L, which were measured at sites 7 and 2 respectively (Table 88). In the sediment samples, the values ranged between 21.54 (± 0.02) and 39.36 (± 0.01) µg/g, measured at sites 4 and 7 respectively. The mean body concentrations ranged between 20.01 (± 0.13) µg/g, which was measured at site 7, and 117.33 (± 0.10) µg/g, which was measured at site 1. One-way ANOVA indicated significant spatial differences in the mean water, mean sediment and mean body concentrations of Zn ($p < 0.001$).

TABLE 88: The mean concentrations of Zn measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during spring 2000, n = 5

	Water	Sediment	Body
Site 1	4.30 ± 0.03	21.60 ± 0.02	117.33 ± 0.10
Site 2	12.30 ± 0.03	30.77 ± 0.03	40.25 ± 0.16
Site 3	8.10 ± 0.02	40.0 ± 0.10	100.25 ± 0.12
Site 4	9.30 ± 0.02	21.54 ± 0.10	100.0 ± 0.10
Site 7	1.90 ± 0.02	39.36 ± 0.04	20.01 ± 0.13

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

The summer mean water concentrations measured in the samples from sites 3 and 4 were more or less similar (Table 89), with the values ranging between 3.55 (± 0.03) and 16.65 (± 0.03) µg/L. In the sediments samples, the values ranged between 46.57 (± 0.10) and 100.00 (± 0.03) µg/g, with those obtained from site 4 having a significantly higher mean concentration ($p < 0.001$) than those obtained from the other sites. The mean body concentrations of Zn measured at the different sites also varied significantly ($p < 0.001$), with those from site 7 (105.22 ± 0.10 µg/g) being significantly higher than those from the other sites.

TABLE 89: The mean concentrations of Zn measured in the water (µg/L), sediment and body samples (µg/g) of *P. exigua* from the different sites during summer 2000, n = 5

	Water	Sediment	Body
Site 1	9.95 ± 0.03	81.25 ± 0.10	55.96 ± 0.10
Site 2	8.0 ± 0.10	56.20 ± 0.10	90.73 ± 0.10
Site 3	16.30 ± 0.03	65.0 ± 0.10	108.18 ± 0.10
Site 4	16.65 ± 0.10	100.0 ± 0.03	53.0 ± 0.11
Site 7	3.55 ± 0.10	46.57 ± 0.10	105.22 ± 0.10

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

During autumn, the mean water concentrations of Zn ranged between 6.30 (± 0.03) and 17.80 (± 0.04) µg/L (Table 90), with the values being in the order: site 3 > site 4 > site 2 > site 1 > site 7. In the sediments, the mean concentrations ranged between 20.98 (± 0.10) and 110.46 (± 0.10) µg/g, with the mean concentration from site 4 being significantly higher than those from the other sites ($p < 0.001$). One-way ANOVA showed that the spatial differences in the mean Zn concentrations of the sediment samples were highly significant ($p < 0.001$). The mean body concentrations ranged between 60.17 (± 0.12) and 109.90 (± 0.12) µg/g, with those from sites 3 and 7 being more or less similar. One-way ANOVA indicated significant spatial differences in the mean concentrations of the body samples ($p < 0.001$).

TABLE 90: The mean concentrations (± SE, n = 5) of Zn measured in the water, sediment and body samples of *P. exigua* from the different sites during autumn 2001

	Water	Sediment	Body
Site 1	10.12 ± 0.04	85.91 ± 0.01	61.50 ± 0.12
Site 2	10.95 ± 0.03	67.37 ± 0.04	101.0 ± 0.13
Site 3	17.80 ± 0.04	20.98 ± 0.10	109.77 ± 0.12
Site 4	17.0 ± 0.04	110.46 ± 0.10	60.17 ± 0.10
Site 7	6.30 ± 0.03	61.50 ± 0.04	109.90 ± 0.12

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

The mean Zn concentrations measured during winter 2001 are shown in Table 91. The mean water concentrations ranged between 11.97 (± 0.01) µg/L, which was measured at site 7, and 20.30 (± 0.02) µg/g, which was measured at site 3. One-way ANOVA indicated significant spatial differences in the mean water concentrations of Zn ($p < 0.001$). In the sediments, the mean concentrations ranged between 34.83 (± 0.03) and 119.55 (± 0.02) µg/g, with the mean concentration of the samples from site 4 being significantly higher than the others ($p < 0.001$). The mean body concentrations ranged between 85.75(± 0.13) and 120.35 (± 0.13) µg/g. One-way ANOVA indicated significant spatial differences in the mean body concentrations ($p < 0.001$), and that the body concentrations were significantly higher ($p < 0.001$) than those of the water and sediments at most sites during most seasons, except for the few instances when the sediment concentrations became higher than those of the water and body samples

TABLE 91: The mean concentrations (± SE, n = 5) of Zn measured in the water, sediment and body samples of *P. exigua* from the different sites during winter 2001

	Water	Sediment	Body
Site 1	12.44 ± 0.02	90.25 ± 0.01	98.0 ± 0.13
Site 2	14.87 ± 0.02	69.44 ± 0.10	113.77 ± 0.10
Site 3	20.30 ± 0.02	34.83 ± 0.03	120.35 ± 0.13
Site 4	18.22 ± 0.02	119.55 ± 0.02	85.75 ± 0.13
Site 7	11.97 ± 0.01	71.75 ± 0.10	115.0 ± 0.11

(Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

One-way ANOVA was used to determine whether there were any significant seasonal variations in the mean concentrations of each heavy metal at the different sites. The results showed that the winter 2000 and 2001 concentrations were significantly higher than those measured during the other times of the year ($p < 0.001$). The concentrations of the five heavy metals measured in the water, sediment and body samples were also compared using one-way ANOVA, to determine which metal was accumulated most in the different samples. For the water samples, the results showed that the mean Zn concentrations were significantly higher ($p < 0.001$) than all the other heavy metals except at site 1 during winter 2001, when the Pb concentrations became significantly higher than the other heavy metals ($p < 0.001$), and at site 4 when the mean Pb concentration became significantly higher than the other metals during spring ($p < 0.001$). In the sediment samples, Zn was the heavy metal accumulated most at most sites and during most seasons, except when the mean Pb concentration was significantly higher than the other heavy metals during winter 2000 at site 4 ($p < 0.001$), during spring when the mean Pb concentrations at sites 2 and 4 were significantly higher than the other heavy metals ($p < 0.001$), and during autumn when the Pb concentrations were significantly higher than those of the other heavy metals at site 3 ($p < 0.001$). In the body samples, Zn was the heavy metal most accumulated at all the sites and during all five seasons.

7.4. DISCUSSION

The lower salinity measured earlier at site 1 (Table 9, Chapter 2) may be an indication of strong freshwater inflow from the Lourens River which opens at this site (Taljaard et al., 2000). The higher winter rainfall (Table 4, Chapter 2) may have resulted in the decreased salinity at this site, which in turn may have contributed to the high heavy metal levels in the whole-body samples of *P. exigua* during winter in the present study (Tables 68; 72; 76; 81 & 87).

In the present study, there was a slight reduction in the pH measured during the winter period (Table 67), which may be related to the presence of atmospheric pollutants such as nitrogen oxides, sulphur dioxide, Pb and ozone which have been identified in the Cape Metropolitan area (CMC, 1999). Although there is no monitoring programme or data on the effect of air pollution on the water quality of False Bay (Taljaard et al., 2000), it may be assumed that the presence of these pollutants caused an "acid rain" effect which has been observed in other parts of the world (Parsons & Takahashi, 1973), resulting in the reduced pH values measured in the present study.

According to Darracott & Watling (1975), the water pollutant concentrations fluctuate widely under conditions of variable rainfall. The significant differences in the rainfall measured during the present study (Table 10, Chapter 2) may have contributed to the highly variable concentrations of Cu, Ni and Zn at the different sites (Tables 73; 77 & 87) since, according to Forstner & Wittman (1979), high concentrations of these heavy metals are often seen following high runoff events.

The tissues of starfish have been found to accumulate high levels of heavy metals, and this has been attributed to the lower levels of biotransformation enzymes in these organisms (Anon, 1979). In a previous study (Eisler, 1981), field-collected samples of echinoderms were found to accumulate up to 180 µg/g Cd (dry weight), which is about 16-fold the levels measured in the present study.

The highly variable heavy metal concentrations observed in *P. exigua* in the present study may be related to the high elimination capacity which has been observed in echinoderms previously (Békri & Pelletier, 2003). Another reason for this variation may be related to its algal source of food, which has high sorption capacities for metals (Conti & Cecchetti, 2002; Hashim & Chu, 2003) and which may then contribute to the metal levels in the sea star. A previous study of the echinoderm *Asterias rubens* from the North Sea, (den Besten et al., 2001) showed that seasonal variations in the heavy metal concentrations of these organisms may be related to the reproductive cycle, since the onset of gametogenesis may result in the transport of metals accumulated in the body of the sea star to the gonads. Although the biology of *P. exigua* from False Bay is not clearly understood, the reproductive cycle may have influenced the heavy metal levels in the present study.

Eisler (1981) previously reported that the Cu levels in echinoderms were often lower than those of other marine invertebrates. The results of the present study showed, however, that the Cu concentrations of *P. exigua* had reached a maximum level of 41.75 µg/g, which was higher than the maximum levels recorded for the limpets (undetectable to 20.00 µg/g) (Table 44, Chapter 5), the mussels (not detectable to 5.00 µg/g) (Figure 62, Chapter 6) and the barnacles (not detectable to 40.25 µg/g) (Table 25, Chapter 3).

According to Freeman et al., (2001), the increase in water temperature and upwelling currents seem to be important factors in the spawning of asteroids, with spawning coinciding with warmer temperatures. Following spawning, gonadal development occurs throughout the winter, with the reproductive peak reached in late spring (Freeman et al., 2001). In the present study, the decrease in the heavy metals during summer may be related to the spawning process, while the increased concentrations during winter may be related to gonadal development which is often accompanied by metal accumulation (Regoli, 1998).

7.5. CONCLUSION

Spatial and temporal variations were observed in the heavy metal content of *P. exigua,* which may be related to its high elimination capacity, although other factors such as salinity, pH, rainfall and the reproductive cycle may have played a role. Since echinoderms frequently remain immobile for long periods, the heavy metal levels measured in their bodies may reflect integrated contamination levels. As bottom dwellers, it may be that the echinoderms are closely associated with sediments, thus making them susceptible to the heavy metals which have accumulated in the sediments. It may be concluded that the echinoderm *P. exigua* can be a valuable biomonitor of heavy metal contamination and may be useful in biomonitoring spatial and temporal contamination trends in the intertidal zone.

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CHAPTER 8 – CADMIUM UPTAKE, DISTRIBUTION AND ELIMINATION BY MARINE INVERTEBRATES FROM THE FALSE BAY INTERTIDAL ZONE

8.1. INTRODUCTION

The increase in the occurrence of heavy metals through the disposal of effluents in the marine environment, has resulted in many studies on the toxicity and uptake of these elements by marine organisms (Kureishy & D'Silva, 1993). Since these organisms are able to accumulate heavy metals such as Cd to high levels, the possible cumulative effects of the ingestion of sub-lethal doses of Cd on humans eating contaminated shellfish are cause for concern (Carpene & George, 1981).

Various metal accumulation and storage mechanisms have been observed among the invertebrates (Newell, 1979), as well as varying degrees of specific metal distribution in the different organs, with previous authors (Baudrimont et al., 1999) proposing that metal distribution was dependent on the route of uptake, and on the properties of the biological barriers (i.e. giils, mantle and gut wall) that separate organs from the surrounding medium. Many studies on the accumulation of trace metals by marine invertebrates assume that a simple linear relationship exists between metal concentration in the water and in marine organisms (Chan, 1988). However, the net accumulation of the metals that are taken up depend on various factors, such as the relative rates of metal excretion and storage in detoxified form (Chan, 1988). According to Rainbow et al. (1990), the accumulation of metals in marine organisms involves the compartmentalization of the metals to render them unavailable metabolically. In bivalves, the accumulated metals are "detoxified" by storing them in granules which are found in the kidney and which are excreted via urine.

The loss of metals by organisms is defined in terms of the biological half-life of a particular metal (Cunningham, 1979), and is affected by the total body concentrations. The biological half-life is the time required for half of the accumulated metal to be lost as a result of biological processes (Cunningham, 1979), and has been found to be longer in animals whose tissue residue is higher. According to Okazaki & Panietz

(1981), metal elimination may be related to the deposition or binding characteristics of metals in the organs, and may occur through the elimination of mucus or faeces, or through the elimination of amoebocytes, also called diapedesis (Newell, 1979).

Crustaceans detoxify the accumulated metals in granules which are found in the midgut gland and which are released through defaecation (Rainbow et al., 1990). From the point of view of estimating the probable effects of bioaccumulation, it is essential to obtain a clear understanding of organ distribution of the pollutants in question (Anon., 1979). Studies on the distribution of contaminants over time in the individual tissues provide information on the relative response of each tissue and the possible dominant route of uptake of contaminants (Anon, 1980). To understand the biological effects of heavy metals, there is a need for individual tissue analysis, so that effects may be correlated to with the metal concentrations.

Unlike hydrocarbon pollution which produces visible buildup in the environment, the relatively soluble heavy metals may accumulate unnoticed, to high toxic levels (Darracott & Watling, 1975). There is increasing recognition that Cd is highly toxic even at low concentrations (Webb, 1979), and that it can be integrated and become concentrated in food chains due to its long-term persistence in soils and its rapid uptake and accumulation by plants and animals. Phytoplankton, which are a source of food for invertebrates, tend to accumulate large amounts of Cd (Nassiri et al., 1997), thus resulting in its transfer to higher trophic levels. Thus, establishing how marine organisms such as the invertebrates accumulate Cd would assist in understanding the routes of transfer through the marine ecosystem.

There is a lack of information on the mechanism of Cd uptake, accumulation in certain organs, and its elimination from the tissues by marine invertebrates from False Bay. This part of the study was undertaken to address such issues, and to contribute to an understanding of these processes. The uptake, distribution and elimination of Cd was studied under laboratory conditions in order to determine whether interspecific differences existed in the dynamics of Cd.

8.2. MATERIALS AND METHODS

8.2.1. Exposure experiments

Exposure experiments were carried out between September 2000 and February 2001. Forty individuals of each of the species *Oxystele tigrina, Choromytilus meridionalis, Patella oculus* and *Patiriella exigua* were collected from the rocky shore. The barnacle *T. serrata* was excluded from this part of the study because of its high mortality under laboratory conditions during trial experiments performed earlier. The specimens were collected from an undeveloped and relatively uncontaminated area bordering the Cape Peninsula National Park, near site 7 (Figure 1, Chapter 2). The animals were selected to be within a narrow size range, under the assumption that organisms within a certain class size were more or less the same age (Anon, 1980). Thus, size-related differences in heavy metal accumulation (Bourgoin, 1990) were circumvented by choosing individuals of a standard size. The different size ranges were 32-40 mm shell length for the periwinkle *O. tigrina,* 55-65 mm shell length for the mussel *C. meridionalis,* 31-37 mm shell length for the limpet *P. oculus,* and 40-50 mm diameter for the sea star *P. exigua.*

The field-collected organisms were first acclimated to the laboratory conditions by placing them in uncontaminated seawater for 2 days before the start of the exposure period. The aim of holding the animals in the seawater was to allow the complete depuration of the gut contents, which, if not done, would influence whole-body analytical results (Anon., 1980) and thus contribute to variations in the heavy metal concentrations. For the exposure experiments, the organisms were randomly placed in three 50-L glass aquaria containing 10 litres of aerated seawater, with small rocks and fronds of green and brown algae to provide attachment. The aquaria were kept in a climate room which was maintained at 17 ± 1 °C under a 12 h: 12 h light: dark regime. The two treatment groups were exposed to $CdCl_2$ for 14 days, with one group exposed to a concentration of 200µg/L $CdCl_2$ and the other to 400 µg/L $CdCl_2$. These concentrations had previously been determined as sublethal (Regoli et al., 1991). The control organisms were kept in clean aerated seawater for the duration of the exposure period.

Before the start of the exposure, three individuals of each species were sampled from each of the experimental groups to determine the initial Cd concentrations. The animals were not fed for the duration of the exposure period, and the media were changed every third day. The clean seawater used to replace the media was collected from the less impacted and relatively "uncontaminated" Cape Peninsula National Park near site 7 (Figure 1, Chapter 2). The pH of the media was measured using a pH meter, by sampling 5ml of the media every third day before the media change was carried out. Mortality was checked daily, and the dead organisms were removed. The mussels were considered to be dead if the shell remained agape after tapping (Harrison et al., 1983), while the gastropods were considered dead when they failed to retract their feet upon prodding.

It must be noted that the exposures in the present study were carried out in a static-flow system. Although a continuous-flow system is desirable in long-term studies, it was found previously that there was no significant difference in the results measured in static and constant-flow systems in short-term studies on the mussel *Mytilus edulis* (Scott & Major, 1972).

8.2.2. Decontamination experiments

These experiments were carried out to determine how much of the accumulated Cd would be lost by the exposed animals after a week's decontamination. After the 14-day exposure, the surviving animals were removed from the exposure media, rinsed and then placed in clean seawater to allow them to depurate for one week. The seawater used for the decontamination experiments was collected from the Cape Peninsula National Park, and was changed once during the decontamination week. At the end of this period, three individuals of each species were sampled from each of the experimental groups and analysed for Cd.

8.2.3. Heavy metal analysis

During the exposure period, the levels of the dissolved Cd taken up by the animals, as well as the distribution of Cd in the different body organs (whole soft tissues, foot muscle, digestive gland, gills, shell and kidney, where applicable) were monitored. This was done by random sampling of 5 individuals of each species from each treatment group at the start of the experiment (that is, day 0), and on the 3rd, 7th, 10th and 14th days of exposure. The animals were killed by freezing them at –20 °C, and then kept in the freezer until further analysis. The specimens were then thawed, the soft tissues separated from the shells, and the different organs (foot muscle, digestive gland, gills) were dissected out. The samples of each type of organ were pooled, oven-dried at 60°C for 48 h and then ground to a powder using a pestle and mortar. Samples (0.2 – 0.3 g) were then acid-digested as described previously (Chapter 2, p. 18). The digests were analysed for Cd using the AAS method as described in Chapter 2 (p. 18), and the metal concentration was expressed as µg/g dry weight. The rate of net Cd accumulation during the exposure period, and the rate of Cd loss during the decontamination week was calculated and expressed as µg/g/day.

8.2.4. Statistical analysis

The comparison of the Cd burden in the different body organs of each species was carried out using one-way ANOVA. One-way ANOVA was also used to compare the Cd concentrations of the control and treatment groups of each species. The t-tests were used to compare the differences in the Cd concentrations measured on the initial and last day of the exposure period for each organ type.

8.3. RESULTS

8.3.1. Mortality

The water pH recorded during the exposure period was 7.79 ± 0.10 for the control, 7.60 ± 0.04 for the 200 µg/L and 7.02 ± 0.07 for the 400 µg/L media. The mean cadmium concentrations measured in the control seawater ranged between undetectable levels and 2.00 (± 0.12) µg/L. A visible sign of Cd toxicity in the two

tanks containing the toxicant was increased mucus secretion by the exposed organisms, resulting in media foaming. There was increased frothing at the higher exposure concentration. No deaths occurred in the controls and 200 µg/L groups, while there was 17.5% mortality among *P. oculus*, 10% mortality among O. *tigrina,* 8% mortality among *C. meridionalis* and 5% among *P. exigua* from the 400 µg/L group (Table 92). Most of the deaths among *P. oculus* and *O. tigrina* occurred from day 7 onwards, while those among *P. exigua* and *C. meridionalis* occurred from day 10 of the exposure period.

TABLE 92: Mean mortality (numbers) of the different species of the group exposed to 400 µg/L $CdCl_2$ (n = 40)

Exposure period (days)	Mortalities (numbers)			
	O. tigrina	*P. oculus*	*C. meridionalis*	*P. exigua*
0	0	0	0	0
3	0	0	0	0
7	1	2	0	0
10	2	2	1	1
14	1	3	2	1
Total	4	7	3	2
% mortality	10	17.5	7.5	5

8.3.2. Cd uptake and organ distribution

8.3.2.1. Cd concentrations in the organs of *Oxystele tigrina*

The organ-specific Cd accumulations measured for *O. tigrina* are presented in Figures 3-5. In the control organisms (Figure 3), the digestive gland had a significantly higher mean Cd concentration ($p < 0.05$) compared to the other tissues (Appendix 1). There was a progressive decrease in the Cd concentrations of all the organs of the control group. At the end of the exposure period, the mean Cd concentration of the soft tissues of the control group of *O. tigrina* had decreased from 6.45 (± 0.15) to 4.10

(± 0. 0.12) µg/g dry weight. The mean Cd concentration of the shells decreased from 2.33 (± 0.10) to1.45 (± 0. 0.16) µg/g, the foot concentrations decreased from 14.84 (± 0.10) to 10.22 (± 0.12) µg/g and the gland concentration decreased from 23.10 (± 0.14) to 19.45 (± 0. 0.14) µg/g.

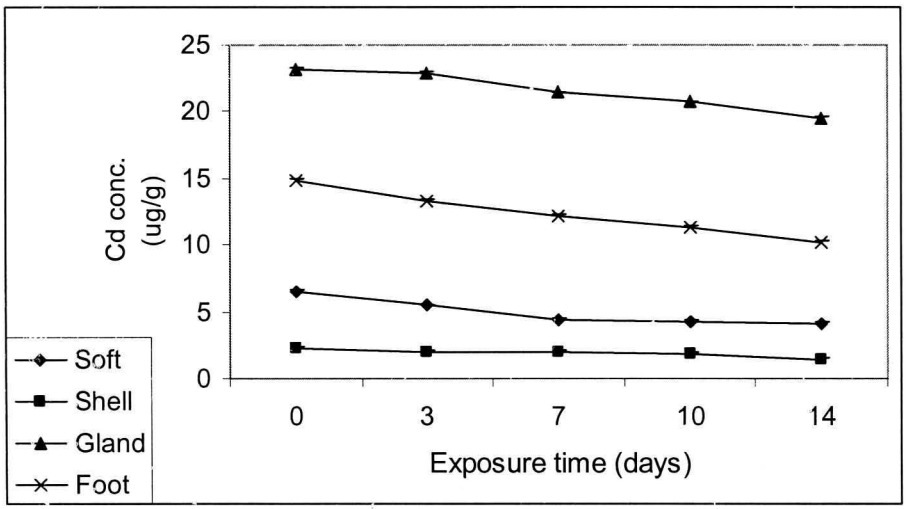

Figure 3: Cd concentrations (µg/g) at the different exposure time intervals in the different organs of *O. tigrina* from control organisms (n = 5 at each time interval)

In the *O. tigrina* specimens which were exposed to 200 µg/L, the mean Cd concentration of all the tissues increased throughout the exposure period (Figure 4). The soft tissue mean concentration increased from 7.38 (± 0.10) to 64.00 (± 10.60) µg/g (Appendix 2). The shell mean concentration increased from 2.05 (± 0.12) to 9.15 (± 0.20) µg/g, the foot mean concentration increased from 16.00 (± 0.11) to 34.00 (± 0.13) µg/g, while the gland mean concentration increased from 22.90 (± 0.12) to 48.28 (± 0.10) µg/g. The Cd accumulation rate in the soft tissues increased between days 3 and 7, from 3.1 to 5.6 µg/g/day, resulting in a higher mean Cd concentration (45.60 ± 0.03 µg/g) on day 7. Between day 7 and day 14, the rate of Cd accumulation continued to increase in the soft tissues, resulting in significantly higher Cd concentrations (64.00 ± 0.14) µg/g) in the soft tissues on the last day of exposure. In the digestive gland, the increase in the rate of Cd accumulation from 0.9 to 2.1

µg/g/day between days 7 and 10 resulted in the Cd concentration becoming nearly double the initial concentration (23.10 ± 0.12 µg/g).

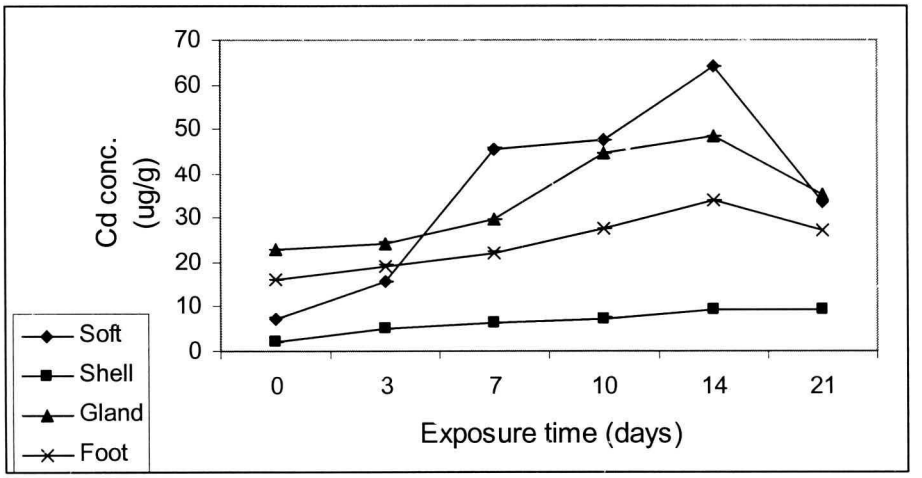

Figure 4: Cd concentrations (µg/g) at different time intervals in the different organs of *O. tigrina* exposed to 200 µg/L $CdCl_2$ for 14 days and allowed to decontaminate for 7 days (n = 5 at each time interval)

By the end of the exposure period, the soft tissues had accumulated more than 9-fold the initial concentration (6.45 ± 0.20 µg/g) of Cd, while the digestive gland had double the initial Cd concentration. The Cd accumulation in the shell and foot muscles increased gradually and progressively throughout the exposure period, occurring more or less linearly. By the end of the exposure period, the shell mean Cd concentration had increased by more than 3-fold the initial amount (2.33 ± 0.12 µg/g), while that of the foot muscle had more than doubled.

In the *O. tigrina* specimens which were exposed to 400 µg/L (Figure 5), the mean Cd concentrations increased progressively throughout the exposure period. Between day 0 and day 3 (Appendix 3), the Cd accumulation rate (15.9 µg/g/day) of the soft tissues increased rapidly, resulting in slightly higher Cd concentrations compared to the other tissue types on the 3rd day. Between days 3 and 10, the accumulation rate in the soft tissues increased steadily and linearly, but it increased rapidly again between days 10

and 14, resulting in a significantly higher mean Cd concentration (122.00 ± 0.0.13) µg/g on the last day of exposure. By the end of the exposure period, the soft tissues had accumulated about 18-fold the amount of the initial concentration. The mean Cd concentrations of the digestive gland were slightly higher than those of the other tissue types between day 0 (27.25 ± 0.13 µg/g) and day 3 (42.86 ± 0.10 µg/g) of the exposure period. By the end of the exposure period, the mean Cd concentration of the digestive gland (72.00 ± 0.13 µg/g) was more than triple that of the initial concentration. The shell concentration increased more or less linearly until it was 13-fold the initial concentration, while that of the foot muscles increased by about triple the initial Cd concentration. On day 14, the mean Cd concentrations of the two exposure groups were in the order: soft tissues > digestive gland > foot muscle > shell.

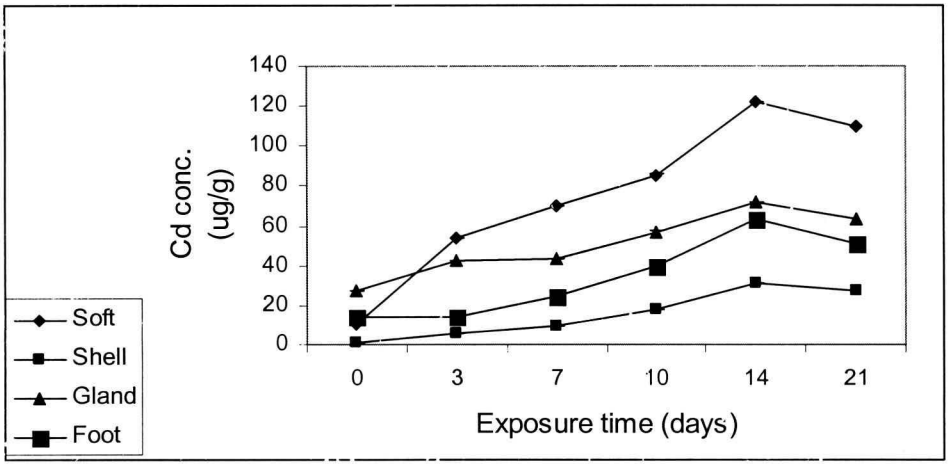

Figure 5: Cd concentrations (µg/g) at different time intervals in the different organs of *O. tigrina* exposed to 400 µg/L $CdCl_2$ for 14 days and allowed to decontaminate for 7 days (n = 5 at each time interval)

During the decontamination week, the Cd concentration in the various organs decreased. The shells of the group exposed to 200 µg/L lost a higher percentage (89%) of the accumulated Cd at a rate of about 0.9 µg/g/day, followed by the soft

tissues (53%) at a rate of about 4.4 µg/g/day, and then the digestive gland (52%) at a rate of about 1.9 µg/g/day (Figure 4). The percentage of Cd lost by the foot muscle (35%) of the 200 µg/L group was the lowest. The proportion and rates of Cd loss from the organs of the higher exposure concentration was lower, with the foot muscles losing 25% of the accumulated Cd at 1.8 µg/g/day, the digestive gland losing 17% at1.2 µg/g/day, the shells losing 14% at 0.6 µg/g/day, and the soft tissues losing 10% at 1.7 µg/g/day (Figure 5).

One-way ANOVA was used to compare the Cd concentrations in the different organs of the control and exposed groups. The results showed that in the soft tissues and gland samples, the mean Cd concentrations measured in the higher concentration group were significantly higher than those of the unexposed group and the group exposed to the lower concentration (200 µg/L) ($p < 0.001$) throughout the exposure period. In the shells, the mean Cd concentration of the organisms exposed to the higher concentration (400 µg/L) was significantly higher than those of the other experimental groups from the 3rd day until the end of the exposure period ($p < 0.05$). In the foot muscles, the mean Cd concentrations of the organisms from the higher exposure concentration were significantly higher than those of the other experimental groups from the 7th until 14th day ($p < 0.001$).

8.3.2.2. Cd concentrations in the organs of *C. meridionalis*

The Cd concentrations measured in the control and the Cd-exposed groups of the mussels are shown in Figures 6 – 8. In the control group (Appendix 4), the mean Cd concentration of the kidneys which was measured on day 0 (10.50 ± 0.10) µg/g was slightly higher than those of the other organs (Figure 6). Although there was a progressive loss of Cd by all the organs, the rate of Cd loss in the kidneys (0.9 µg/g/day) between day 0 and day 3 was relatively faster than the rate of loss from other organs. By day 14, the mean Cd concentration in the soft tissues of *C. meridionalis* had decreased from 9.60 (± 0.10) to 6.30 (± 0.10) µg/g, the shell concentration had decreased from 7.41 (± 0.11) to 3.99 (± 0.12) µg/g, and that of the gills had decreased from 0.90 (± 0.12) to below detection levels.

The gland mean concentration decreased from 10.00 (± 0.11) to 5.20 (± 0.10) µg/g, while the mean concentration of the kidney decreased from 10.50 (± 0.10) to 5.07 (± 0.17) µg/g. The mean Cd concentrations measured in the day 14 samples were in the order: soft tissues > digestive gland > kidney > shell > gills.

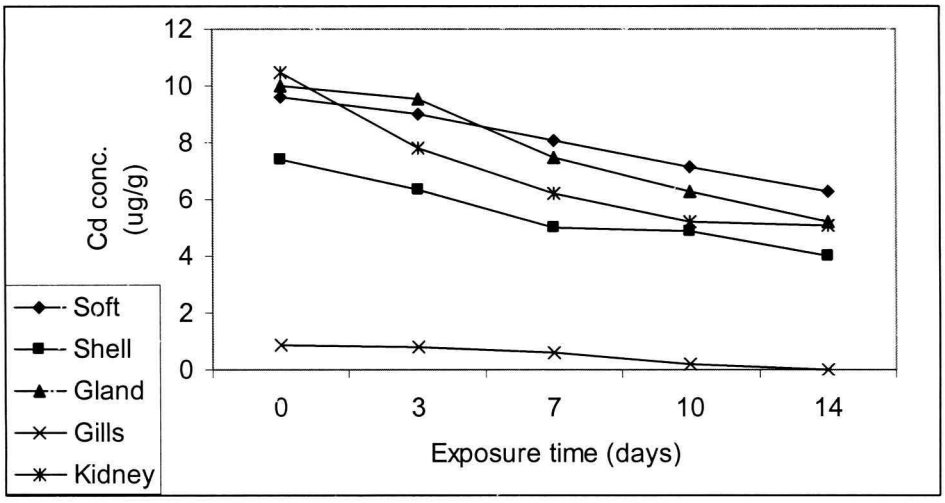

Figure 6: Cd concentrations (µg/g) at different time intervals in the different organs of *C. meridionalis* organisms in the control group (n = 5 at each time interval)

In the mussels which were exposed to 200 µg/L, the mean Cd concentration measured in the kidneys was slightly higher than those of the other organs (Figure 7), and remained so while gradually increasing more or less linearly until day 10 (Appendix 5). Between days 10 and 14, however, the rate of Cd accumulation in the soft tissues increased rapidly, from about 2.4 to 4.3 µg/g/day, resulting in a significantly higher ($p < 0.001$) mean Cd concentration in the soft tissues (69.50 ± 0.10 µg/g) on the last day of exposure, which led to an increase of more than 6-fold the initial concentration of the soft tissues. At the end of the exposure period, the mean Cd concentration of the kidneys was about six times that of the initial concentration. The shell and gland samples accumulated Cd more or less linearly, resulting in about triple the amount of the initial concentrations by day 14.

At the end of the exposure period, the Cd concentrations were in the order: soft tissues > kidney > digestive gland > shell = gills.

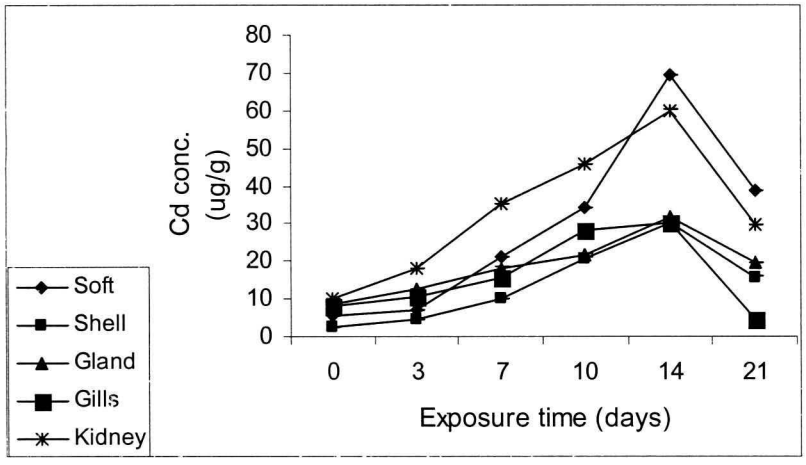

Figure 7: Cd concentrations (µg/g) at different time intervals in the different organs of *C. meridionalis* exposed to 200 µg/L $CdCl_2$) for 14 days and allowed to decontaminate for 7 days (n = 5 at each time interval)

In the mussels which were exposed to the higher concentration (400 µg/L), the Cd accumulation rate of the soft tissues increased sharply between days 3 and 7 (Figure 8), from 3.8 to 18.3 µg/g/day, resulting in the Cd concentrations increasing nearly 16-fold (Appendix 6). From day 7 onwards, the Cd concentration in the soft tissues increased more or less linearly, to reach a significantly high concentration (197.00 ± 0.41) µg/g at the end of the exposure period. The kidneys accumulated Cd linearly from the first day of exposure to the 10th day, resulting in the Cd concentrations being eight times that of the initial concentration by day 14. The gills accumulated Cd at a faster rate between days 0 and 3, at a rate of 4.8 µg/g/day). The Cd accumulation rate in the gills decreased between days 3 and 7, then increased again between days 7 and 14.

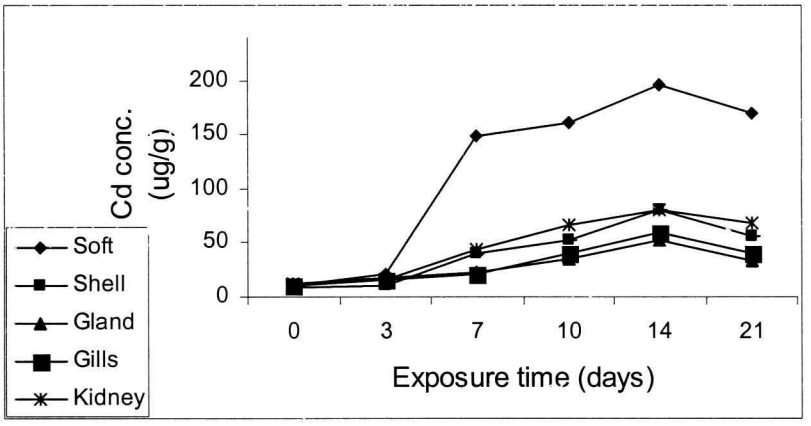

Figure 8: Cd concentrations (µg/g) at different time intervals in the different organs of *C. meridionalis* exposed to 400 µg/L $CdCl_2$) for 14 days and allowed to decontaminate for 7 days (n = 5 at each time interval)

By the end of the exposure period, the mean Cd concentration of the gills (60.00 ± 0.12 µg/g) was about 66 times the initial concentration (0.90 ± 0.15 µg/g). The shells and the digestive gland had slower accumulation rates, with Cd values increasing linearly throughout the exposure period. By the end of the exposure period, the mean Cd concentration of the shell (80.00 ± 0.13 µg/g) was about 11 times the initial concentration (8.70 ± 0.11 µg/g), while that of the digestive gland (52.17 ± 0.13 µg/g) was about six times that of the initial concentration (12.50 ± 0.12 µg/g). On day 14, the mean Cd concentrations were in the order: soft tissues > shell = kidney > gills > digestive gland. One-way ANOVA showed that there were significant differences between the mean Cd concentrations of the control and exposed groups, with concentration 2 values being significantly higher than those of the other two groups in all the organs, from day 7 until the end of the exposure period ($p < 0.001$).

During the depuration week, the mean Cd concentration in the gills of the 200 µg/L group (Figure 7) decreased from 30.00 (± 0.13) to 4.70 (± 0.13) µg/g, reflecting Cd loss at a rate of about 3.6 µg/g/day. The shell mean concentration decreased from 30.00 (± 0.13) to 15.69 (± 0.16) µg/g at a rate of 2.0 µg/g/day.

The mean concentration of the kidneys decreased from 60.00 (± 0.15) to 29.80 (± 0.12) µg/g at a rate of 4.3 µg/g/day. The mean concentration of the digestive gland decreased from 31.84 (± 0.10) to 19.70 (± 0.13) µg/g at a rate of 1.7 µg/g/day, while that of the soft tissues decreased from 69.50 (± 0.10) to 38.70 (± 0.12) µg/g at a rate of 4.4 µg/g/day. In the group that was exposed to 400 µg/L (Figure 8), the mean Cd concentration in the gills decreased from 60.00 (± 0.12) to 40.06 (± 0.20) µg/g at a rate of 2.9 µg/g/day. The shell mean concentration decreased from 80.00 (± 0.13) to 55.60 (± 0.17) µg/g at a rate of 3.5 µg/g/day. In the kidneys, the mean Cd concentration decreased from 80.00 (± 0.15) to 67.79 (± 0.13) µg/g, at a rate of 1.7 µg/g/day. The mean Cd concentration of the digestive gland decreased from 52.17 (± 0.13) to 33.69 (± 0.11) µg/g at a rate of 2.6 µg/g/day, while that of the soft tissues decreased from 197.00 (± 0.41) to 169.70 (± 0.12) µg/g at a rate of 3.9 µg/g/day.

8.3.2.3. Cd concentration in the organs of *P. oculus*

The Cd concentrations measured in the whole soft tissues and shells of the limpets from the control and the Cd-exposed groups are shown in Figures 9 – 11. The soft tissue mean Cd concentrations of the control organisms (Figure 9) were significantly higher ($p < 0.001$) than those of the shells (Appendix 7). The mean Cd concentrations decreased gradually in the two organs. At the end of the exposure period, the Cd concentration of the soft tissues of *P. oculus* had decreased from 20.08 (± 0.15) to 14.38 (± 0.11) µg/g, while the shell concentration decreased from 2.18 (± 0.15) to 0.42 (± 0.11) µg/g.

In the limpets which were exposed to 200 µg/L, the mean Cd concentrations increased in both the soft tissues and shells (Figure 10). The accumulation rates (1.7 µg/g/day) in the soft tissues were more or less linear between days 0 and 7 (Appendix 8), and then increased rapidly from 1.7 to 14.7 µg/g/day between day 7 and 10, resulting in a mean Cd concentration which was nearly 6-fold the initial concentration (19.60 ± 0.12 µg/g) on day 14. The shells accumulated Cd at a rapid rate initially, with the uptake rate remaining more or less linear between day 3 and day 7, and then doubling

between day 7 and 10, resulting in a final mean concentration which was 19 times the initial concentration. At the end of the exposure period, the soft tissue concentration (110.04 ± 0.13) µg/g was double that of the shells (51.67 ± 0.12 µg/g), and was more than 5-fold the initial soft tissue mean Cd concentrations. Following the decontamination period, the mean Cd concentrations in the soft tissues of the *P. oculus* decreased from 110.00 (± 0.13) to 65.77 (± 0.12) µg/g while the shell mean concentration decreased from 51.67 (± 0.12) to 32.19 (± 0.14) µg/g.

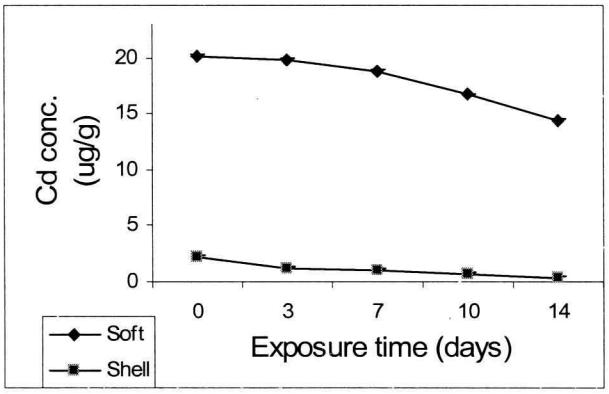

Figure 9: Cd concentrations (µg/g) at different time intervals in the soft tissues and shells of *P. oculus* from the control group (n = 5 at each time interval)

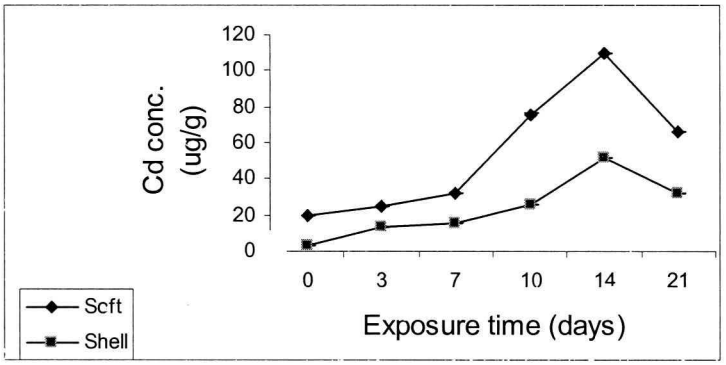

Figure 10: Cd concentrations (µg/g) at different time intervals in the soft tissues and shells of *P. oculus* exposed to 200 µg/L $CdCl_2$) for 14 days and allowed to decontaminate for 7 days (n = 5 at each time interval)

In the organisms which were exposed to 400 µg/L (Figure 11), the soft tissues and shells accumulated Cd linearly between days 0 and 7 of the exposure period (Appendix 9). Between days 7 and 10, there was a rapid accumulation of Cd by the soft tissues of *P. oculus*, from an accumulation rate 4.9 to 31.3 µg/g/day. This was followed by a slower rate of accumulation between days 10 and 14. By day 14, the mean Cd concentration of the soft tissues (140.11 ± 0.19 µg/g) was more than double that of the shells (69.90 ±0.21 µg/g) and was significantly higher ($p < 0.001$) than that of the shells. Following decontamination, the soft tissue mean concentration in the soft tissues of this group lost 21.2 % of the accumulated Cd, while the shells lost 21.7 %. One-way ANOVA showed that the mean Cd concentrations in the group which was exposed to 0.4 mg/L were significantly higher that those of the other two groups from day 7 onwards, in both the soft tissues and shells ($p < 0.001$).

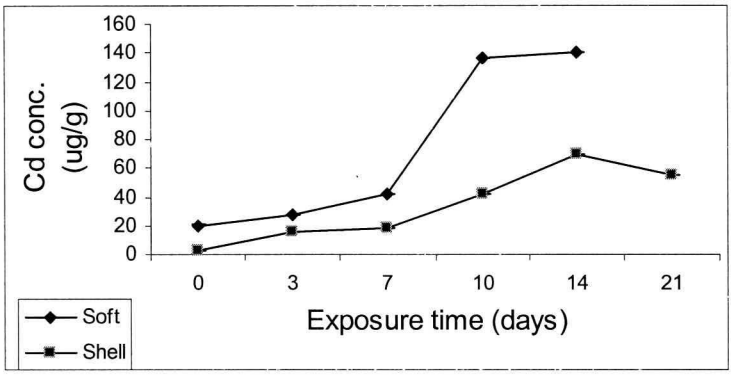

Figure 11: Cd concentrations (µg/g) at different time intervals in the soft tissues and shells of *P oculus* exposed to 400 µg/L $CdCl_2$) for 14 days and allowed to decontaminate for 7 days (n = 5 at each time interval)

8.3.2.4. Cd concentration in the seastar *P. exigua*

The whole-body Cd concentrations which were measured in the control and exposed seastars are shown in Figure 12. While the mean Cd concentrations of the control individuals decreased gradually during the exposure period (Appendix 10), those of the Cd-exposed groups increased gradually and linearly. By day 14, the group which was exposed to 200 µg/L had accumulated 3.93 µg/g Cd while the group which was exposed to 400 µg/L had accumulated 6.37 µg/g Cd, with the mean Cd concentrations of the 400 µg/L group being slightly higher than those of the control and 200 µg/L group. During the decontamination week, the *P. exigua* which were exposed to 200 µg/L lost all of the accumulated Cd, while the group expose to 400 µg/L lost 68 %. One-way showed significant difference between the mean Cd concentrations of the control and 0.4 mg/L group from day 7 until the end of the exposure period ($p < 0.05$).

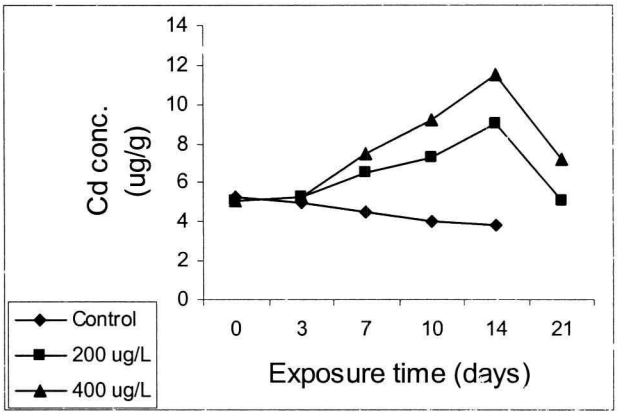

Figure 12: Cd concentrations (µg/g) at different time intervals in the whole-body of *P. exigua* in the control and exposed individuals allowed to decontaminate for 7 days (n = 5 at each time interval)

8.3.3. Comparison of Cd concentrations of the control and exposed organisms of the different species after depuration

At the end of the decontamination period, the organs of the individuals which were exposed to 400 µg/L specimens had significantly higher mean Cd concentrations ($p < 0.001$) compared to those of the control and group which was exposed to 200 µg/L, in all the species under investigation (Figures 13- 15). There were, however, no significant differences in the mean Cd concentrations among the experimental groups of *P. exigua* ($p > 0.05$).

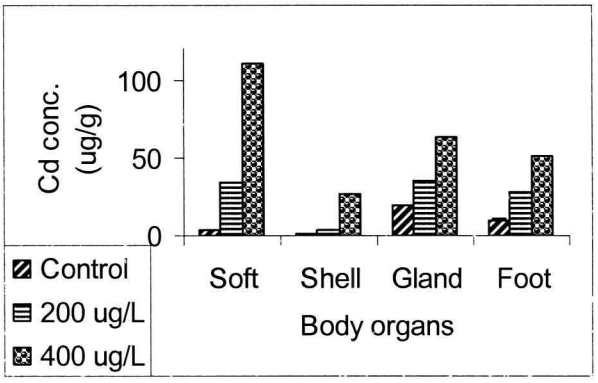

Figure 13: Comparison of the Cd concentrations (µg/g) in the different body organs of specimens of the control and exposed groups of *O. tigrina* following decontamination (n = 5)

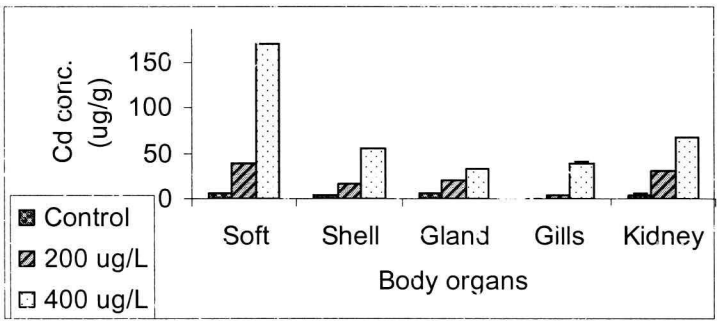

Figure 14: Comparison of the Cd concentrations (µg/g) in the different body organs of specimens of the control and exposed groups of *C. meridionalis* following decontamination (n = 5)

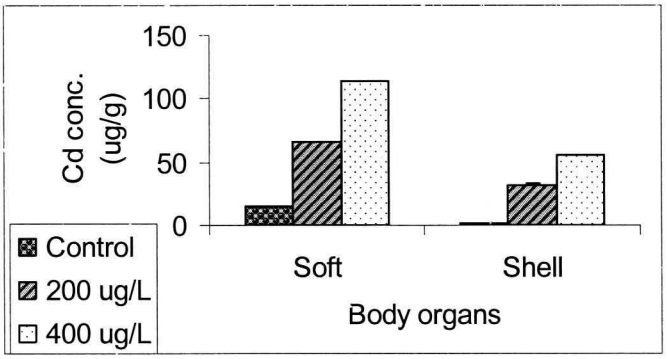

Figure 15: Comparison of the Cd concentrations (µg/g) in the different body organs of specimens of the control and exposed groups of *P. oculus* following decontamination (n = 5)

8.3.4. Comparison of the percentage Cd accumulated by the various organs of the different species

The amount of Cd accumulated by each organ in the different species was calculated by finding the difference between the day 0 and day 14 mean concentrations, and expressing it as a percentage of the day 14 concentration. Among the soft tissues of the two Cd-exposed groups (Tables 93 & 94), those of *C. meridionalis* had the highest percentage of accumulated Cd, followed by *O. tigrina*, *P. oculus* and the lastly by *P. exigua.* Among the shells of the group which was exposed to 200 µg/L, those of *P. oculus* accumulated the highest percentage of Cd, followed by *C. meridionalis* and then by *O. tigrina,* while in the group which was exposed to 400 µg/L it was the shells of *O. tigrina* which accumulated the highest Cd, followed by *P. oculus* and lastly by *C. meridionalis.* The digestive glands of *C. meridionalis* of both groups accumulated more Cd than those of *O. tigrina*.

TABLE 93: Percentages of Cd accumulated by the organs of the different species exposed to 200 µg/L of $CdCl_2$

	O. tigrina	*C. meridionalis*	*P. oculus*	*P. exigua*
Soft tissues	88.5%	92%	82.2%	43.7%
Shells	77.6%	92%	94.8%	_
Gland	52.6%	72.7%	_	_
Foot	53%	_	_	_
Kidney	_	83%	_	_
Gills	_	73%	_	_

TABLE 94: Percentages of Cd accumulated by the organs of the different species exposed to 400 µg/L of $CdCl_2$

	O. tigrina	*C. meridionalis*	*P. oculus*	*P. exigua*
Soft tissues	91.4%	95%	85.6%	55.4%
Shells	96.7%	89%	95.6%	_
Gland	62.2%	76%	_	_
Foot	78.3%	_	_	_
Kidney	_	85%	_	_
Gills	_	83%	_	_

8.4. DISCUSSION

In the present study, the gills of the mussel *C. meridionalis* were found to accumulate 4 times and 6 times the initial Cd concentrations of the 200 µg/L and 400 µg/L groups respectively (Figures 7 & 8). This is in agreement with the results of previous studies (Regoli et al., 1991; Sidoumou et al., 1997) which found that the gills were the main site of metal uptake in bivalves and were able to accumulate high levels of Cd. The ability of the gills to accumulate as much Cd in the present study may be related to their large surface area (Cunningham, 1979). Earlier work (Roesijadi & Unger, 1993) showed that the Cd uptake by the gills occurred by passive diffusion The accumulation of Cd in the gills of mussels in the present study may also be related to the nature of the bivalve feeding process, during which vast amounts of water are drawn into the mantle cavity, causing the water-borne Cd to settle on the gills, trapped by the mucous covering of the organ (Newell, 1979). The rapid Cd uptake which was exhibited by the gills during the first few days of exposure in the present study (Figures 7 & 8) had also been observed previously (Schulz-Baldes, 1977).

The significant loss of Cd from the gills during the decontamination week (Figure 7) is in agreement with previous studies which found a rapid loss of Cd from the gills when the mussels were moved to a "clean" environment (Sidoumou et al., 1997). Considering that the gills of the 200 µg/L group lost 100 % of the accumulated Cd following decontamination (Figure 7) compared to 39.9 % lost by the group which was exposed to 400 µg/L, it may be speculated that only part of the Cd in the gills of the former group was firmly bound to metallothioneins (MTs)(Regoli et al., 1991). These metal-binding proteins play a role in the sequestration of non-essential metals such as Cd, and thus protect the organism against these toxic metals (Burger et al., 2003).

Previous studies (Blackmore, 2000) have shown that heavy metal uptake and tissue distribution in marine organisms are also affected by the presence or absence of food. Previously, the uptake of Cd in fed mussels found to be higher than in starved individuals (Eisler, 1981), and was linked to the increased water pumping rates in the

presence of food. Since the exposures in the present study occurred in the absence of any food supplement, it may be assumed that the Cd accumulation occurred by passive diffusion from the surrounding water (Andres et al., 2000), and may be lower than what it would have been if the exposed animals had been fed.

In the present study, the soft tissues of the Cd-exposed individuals accumulated Cd to varying degrees during the exposure period. The continued and progressive Cd accumulation for the duration of the exposure period in the present study suggests that Cd was not being regulated to a constant concentration in the bodies of the different species (Rainbow et al., 1990). Generally, the soft tissues accumulated more Cd than the shells, which is in agreement with the findings of previous studies (Herwig et al., 1989). According to Newell (1979), a re-distribution of metals occurs from the superficial sites (i.e. gills, mantle and foot) to the internal organs and internal soft tissues during the uptake and decontamination processes. This observation may explain the variations in the Cd uptake and accumulation rates which were observed in the soft tissues at different intervals of exposure in the present study (Figures 4 & 5; 7 & 8; 10 & 11).

The shells of the Cd-exposed animals in the present study also accumulated Cd to various degrees (Figures 4 & 5; 7 & 8; 10 & 11; Tables 93 & 94), a process that may largely be due to adsorption (Herwig et al., 1989. It has been proposed that marine invertebrates incorporate heavy metals into their calcareous shells by displacing the Ca in the crystalline structure (Chinchon et al., 2000). Herwig et al. (1989) previously found that after three weeks of exposure, the shell concentrations of Cd-exposed organisms reached a plateau, suggesting that Cd accumulation via the adsorption process eventually becomes saturated. Since this was not evident in the present study, it may be assumed that the shell binding sites were not fully saturated by the end of the 14-day exposure period.

The accumulation of Cd by the digestive glands of the exposed organisms in the present study (Figures 4 & 5; 7 & 8) is in agreement with previous findings on the accumulation of Cd in the digestive glands of marine organisms (Andres et al, 2000) and may be an indication that the digestive glands are one of the target organs for metal bioaccumulation, as suggested elsewhere (Nott & Nicolaidou, 1989; Jop et al., 1997; Rainbow, 1997; Domouhtsidou & Dimitriadis, 2000). According to Rainbow (1997), the Cd that is accumulated in the digestive glands becomes bound to MTs.

Although the kidney of the mussel, as an internal organ, was not in direct contact with the metal-spiked medium, it was one of the organs that accumulated high concentrations of Cd in the present study, accumulating up to seven times the initial Cd concentration in the group which was exposed to 400 µg/L, and having the second highest concentration of Cd among the different organs (Figures 7 & 8; Tables 93 & 94). The accumulation of Cd in this organ may be related to its function as one of the main storage organ for Cd (Hutzinger, 1982). Various authors have proposed storage mechanisms for Cd, such as manganese- or zinc-rich kidney granules (Rainbow, 1997), membrane-bound vesicles in the kidney epithelial cells (Moore, 1981), as well as MTs and tertiary lysosomes in the kidney (George, 1983).

Herwig et al. (1989) proposed that, in order to reach the kidney, the Cd must first be transported from the primary sites of uptake (i.e. gills and mantle) by the haemolymph and then taken up into the granules of excretory cells in the kidney (Schulz-Baldes, 1977), where the accumulated Cd is changed from ionic to particulate form. This immobilization of Cd in a chemically inert form may be an internal detoxification mechanism by the animal (Schulz-Baldes, 1977). The time lag that was observed in the Cd accumulation by the kidneys of the mussels which were exposed to 400 µg/L during the first few days of the exposure period (Figure 8) was also found previously by Schulz-Baldes (1977) during the uptake of lead into the mussel kidney, and may be due to the transport of the metal via intervening tissues before the internal organs become exposed to the Cd (Herwig et al., 1989).

In the present study, the rates of Cd accumulation tended to be higher than the rates of elimination, which is in agreement with previous studies (Kullenberg, 1982) which found that contaminants tended to be eliminated more slowly than they are taken up, thus resulting in accumulation. Although the 7-day decontamination period in the present study resulted in some loss of the accumulated Cd, the mean Cd concentrations of the exposed groups were still higher than those of the controls (Figures 13-15), indicating either that the Cd was firmly bound and sequestered by the MTs (Sidoumou et al., 1997), or that a longer decontamination period was required for an increased loss of the accumulated Cd (Regoli et al., 1991). In another study, Regoli et al. (1991) found that marine bivalves lost about 50% of the accumulated heavy metals by the 6th day of a decontamination period, and reached levels similar to those of the controls only by the 15th day. The lower amount of Cd which was eliminated from the organs of the group that was exposed to the higher concentration (400 μg/L) of $CdCl_2$ in the present study may be an indication of the Cd being more tightly bound to MTs in this group, thus suggesting more permanent deposition of the metal at the higher concentration, as suggested by Cunningham (1979).

8.5. CONCLUSION

The selected intertidal species (*O. tigrina; C. meridionalis; P. oculus & P. exigua)* which were exposed to Cd showed an increase in Cd concentrations, with different rates of Cd uptake being observed not only among the different species, but among the different organs of each species as well. The Cd concentrations were highest in the soft tissues or kidney in most of the species after two weeks of exposure. The levels of Cd accumulation reflected the exposure concentrations. Both the shells and internal organs demonstrated a loss of Cd when the exposed animals were moved to a clean environment. Following decontamination, a decrease in the accumulated Cd was observed in all the organs, although the rate of loss differed for each specific organ. The organisms that were exposed to the higher concentration (400 μg/L) lost less of the accumulated Cd than that exposed to the lower concentration (200 μg/L), which

may indicate that Cd was more tightly bound to the tissue compartment in the former group.

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CHAPTER 9- LYSOSOMAL RESPONSES AND HISTOLOGICAL CHANGES IN MARINE INVERTEBRATES AS STRESS BIOMARKERS OF HEAVY METAL POLLUTION

9.1. INTRODUCTION

Overt signs of toxicity in affected organisms are nearly always preceded by biochemical, physiological and morphological changes (Overstreet, 1988). Cellular responses provide great potential for identifying organisms for which conditions have exceeded compensatory mechanisms and which are experiencing stress (Ringwood et al., 1998). The haemolymph cells of marine invertebrates play a role in the transport of heavy metals from the uptake organs, as well as in the metabolism and intralysosomal storage of these metals, thus making them an ideal starting point to investigate generalized cellular damage in marine invertebrates, since many cell types in these animals are rich in lysosomes (Moore, 1985).

Contaminant effects at subcellular level have often been found to be associated with lysosomes (Ringwood et al., 1998), and thus can reveal alterations at an early stage of the response (Regoli, 1992). Since the lysosomal system is a target of many accumulated contaminants (Wedderburn et al., 2000), any adverse changes that occur in lysosomes are significant because they indicate the general pathological condition of the affected organism.

According to Owen (1972), the lysosomal stress response involves partial damage and destabilization of lysosomal membranes. Previous studies (Bayne et al., 1979; Ringwood et al., 1998; Reinecke & Reinecke, 1999) have shown that lysosomal destabilization responses are potentially valuable biomarkers of pollutant stress. Techniques for assessing lysosomal destabilization in earthworms using the Neutral Red Retention assay have been developed (Weeks & Svendsen, 1996) and have also been used previously to monitor the effects of xenobiotics on mussels (Cajaraville et al., 1996; Nicholson, 1999b) and freshwater snails (Svendsen & Weeks, 1994).

The NRR assay is based on the fact that the lysosomes of unstressed cells will retain the dye for a longer duration after uptake, while those of stressed cells will leak the dye into the cytoplasm after a shorter period of time. This technique was chosen because of its wide use and reported sensitivity and relevance to heavy metal-induced effects in marine organisms (Lowe & Pipe, 1994; Ringwood et al., 1998; Nicholson, 1999a; Marigomez & Baybay-Villacorta, 2003). Quantitative techniques have also been used to measure the alterations in cells following contaminant exposure (Lowe & Clarke, 1989). The ability to measure changes in the cells of organisms following exposure, in combination with qualitative estimates of the animal's condition, has the potential to provide more sensitive early indications of toxicity (Lowe & Clarke, 1989).

The aim of this part of the present study was to determine and quantify the changes which occur in the lysosomes of haemolymph cells and cells of the digestive gland epithelia of field-collected and laboratory Cd-exposed organisms. The aim was to relate exposure concentration measurements (AAS) to quantitative histological and subcellular effects of Cd. The use of haemolymph lysosomal changes in marine invertebrates as biomarkers of toxic stress in contaminated water could then enhance and lead to more insight about the mechanism of Cd contamination in marine invertebrates inhabiting the False Bay intertidal zone. The choice of Cd for this part of the study is related to its long-term persistence in ecosystems (Bowen, 1982), its uptake and accumulation into the soft tissues of marine organisms and integration in food chains (Webb, 1979) and its known toxicity and harmful effects on molluscs (Nassiri et al., 1997).

9.2. MATERIALS AND METHODS

9.2.1. Neutral Red Retention (NRR) time assay

The lysosomal responses of four marine invertebrate species from the different sampling sites of the False Bay intertidal zone were determined seasonally in order to establish species-specific and site-related differences. Fifty individuals of each of the species *O. tigrina, C. meridionalis, P. oculus* and *P. exigua* were collected from a relatively clean site adjacent to the Miller's Point Marine Reserve (Figure 1,

Chapter 2). The animal sampling was done at low tide during five seasons, beginning in winter 2000 and ending in winter 2001.

The animals were transported to the laboratory in plastic buckets containing site water. At the laboratory, the haemolymph was withdrawn from the foot muscle (in periwinkles and limpets), the adductor muscle (in mussels) and from the radial arms (in sea stars) using a disposable syringe and needle. This procedure enabled repeated sampling from the same animals without any serious harmful effects. For haemolymph extraction in the mussels, the shell valves were forced apart by inserting a stainless steel scalpel blade between the valves in order to insert the needle. A 20 µl sample of the haemolymph was drawn into the syringe containing an equal volume of Ringer solution of the particular animal (Appendix 11) and 20 µl NRR working solution. The working solution, which was renewed every hour, was prepared by mixing 2.5 ml of the specific animal's Ringer solution with 10 µl neutral red stock solution. The stock solution was prepared by dissolving 20 mg of the powdered dye in 1 ml of dimethyl sulphoxide (DMSO). The haemolymph-Ringer-neutral red solution was placed on a slide and covered with a glass coverslip.

The slide was observed under the light microscope (Nikon) at 400X while scanning randomly for 2 minutes. The total number of cells were counted as well as the number which showed a reddish colour due to the leaking of neutral red from the lysosomes into the cytosol. This was done using a manual (sheep) counter. After each count, the slides were incubated in a humidity chamber to prevent drying out. The slides were scanned at 15-minute intervals until there was evidence of dye loss from the lysosomes, indicated by the cytosol staining red, in nearly 50% of the cells scanned. At this time the scanning intervals were reduced to 2 minutes until more than 50% of the total number of lysosomes were stained. This time, expressed in minutes, was recorded as the NRR time. The mean NRR time was calculated from the measurements of 10 different individuals per species.

9.2.2. Neutral Red lysosomal destabilization assay

For this assay, the NRR assay was modified slightly according to the method used by Ringwood et al. (1998). The haemolymph samples were obtained from field-collected animals in the same way as above and mixed with the neutral red-Ringer solution as previously described. The slides were then incubated in a humidity chamber at room temperature for 1 hour, and thereafter examined with a light microscope under 400X magnification, to determine NR retention. During the slide scanning, the cells whose lysosomes retained NR were scored as stable, while those with NR leaking into the cytoplasm were scored as unstable. A minimum of 50 cells were counted for each slide, and the data were expressed as the lysosomal destabilization index, which is the percentage of cells with destabilized lysosomes per animal sample.

9.2.3. Cell viability

The Eosin Y test was used to test the viability of the haemolymph cell suspension. A 20 µl haemolymph sample was drawn into a syringe containing an equal volume of a 0.2% solution of Eosin Y. The solution was then placed on a slide and a coverslip was applied. The slides were then observed under the light microscope to determine the percentage of cells that had taken up the dye, which was an indication of damaged cells (Lowe & Pipe, 1994). Viable cells with intact membranes, staining light green, can exclude Eosin Y, while damaged or dead cells, staining red, cannot exclude it and rapidly take up the dye.

9.2.4. Laboratory exposures

The Cd exposure experiments were carried out between September 2000 and January 2001. Fifty individuals of each of the 4 species were collected from the Miller's Point Marine Reserve and transported to the laboratory in plastic buckets containing site water. After a 2-day acclimation period in the laboratory, the animals were placed in 50-L aquaria containing aerated seawater and exposed to two different concentrations (200 µg/L and 400 µg/L) of Cd in the form of $CdCl_2$. These concentrations had previously been determined as being sublethal (Regoli et al., 1991).

The animals were kept in a controlled climate room at 17^0 C ± 0.03 under a 12 h: 12 h light: dark regime. The two treatment groups were exposed for 14 days, while the control group was kept in clean aerated seawater. The animals were not fed during the exposure period, and the media were changed every third day. Mortality was checked, and the dead animals removed. At the end of the exposure period, the animals were then decontaminated by placing them in clean aerated seawater for a week. The NRR times, as well as the NR destabilization indices, were determined for each of the groups as described earlier, prior to the exposure and then on the 3rd, 7th, 10th and 14th days of the exposure period, as well as at the end of the decontamination week.

9.2.5. Fixation and histological preparations

The digestive glands of the periwinkles, limpets and mussels were excised and placed in 10% formalin fixative for 24 h. The organs were placed in stainless steel embedding cassettes and rinsed in 50% alcohol for an hour. Thereafter, the organs were dehydrated in a series of successive alcohol solutions and cleared in xylene (Appendix 12). The digestive glands were placed in a series of Paraplast wax treatments, and then embedded in fresh pure wax in stainless steel Tissue-Tek moulds, covered with embedding covers and cooled at 5°C overnight.

9.2.6. Sectioning

Cross sections of the digestive glands were made with a Leica Rotary Microtome to a thickness of 6-8 µm. The sections contained in the wax ribbons were then placed on slides. Two drops of Mayer's egg albumin and glycerol mixture were added to the spread wax ribbons, and the slides were then placed on a slide-drying hot plate at 40°C.

9.2.7. Staining and mounting

The slides were stained with Erlich Hematoxylin and Eosin (H & E). The slides were taken through a series of rinsing, dehydration, staining and clearing steps (Appendix 13). After the clearing step, a few drops of Entellan mounting fluid were quickly placed on each slide and a coverslip was added. The slides were left to dry overnight.

9.2.8. Image analysis

The image analysis system consisted of a colour camera mounted on a light Nikon microscope. The image was displayed on a computer screen and captured using a Leica Qwin image analysis program, which allowed the user to acquire, edit and analyze images. Image analysis of the haemolymph samples obtained from the field-collected specimens and the different treatment groups of the exposure experiments, as well as the histological sections was carried out using the 40X objective. For the haemolymph cells, four fields of view were examined randomly on each set of five slides per treatment per species, and ten measurements of lysosomal sizes were made from each slide. For the histological sections, five measurements were made from each set of five randomly selected sections per slide. Four slides were examined for each species per treatment group. Measurements of the digestive gland tubules were made, using the digestive tubule lumen size / area and epithelium cell height as parameters. For the exposed animals, the percentage epithelial areas of the tubules were also measured at 24h, 48h and 72h intervals to assess the extent of changes during the first few hours of exposure to Cd.

9.2.9. Statistical analysis

One-way ANOVA was used to compare the seasonal and spatial NR retention times obtained for each of the field-collected species. The NR retention times of the control and Cd-exposed animals were also compared using one-way ANOVA. One-way ANOVA was used to determine whether there were any significant differences in the lysosomal destabilization indices of field-collected animals, as well as those of the experimental groups. The lysosomal sizes, digestive gland epithelial heights and percentage epithelial areas of the field-collected individuals and the experimental groups were also compared using one-way ANOVA. Regression analyses were carried out to determine the relationship between the NRR times and the body heavy metal concentrations obtained earlier (Chapter 8), between the body concentrations and lysosomal sizes, and between the percentage epithelial area and body concentrations of the Cd-exposed animals.

9.3. RESULTS

9.3.1. NRR time assays

9.3.1.1. NRR times of field-collected specimens

The NR retention times measured for *O. tigrina* are shown in Figure 16. During winter 2000 (Appendix 14), the mean NR retention times ranged between 56 (± 11.17) and 88 (± 7.50) minutes. The spring NR retention times increased slightly to range between 70 (± 10.09) and 93 (± 10.57) minutes. During summer, there was a reduction in the mean NR retention times, with values ranging between 58 (± 13.25) and 82 (± 8.23) minutes. There was a slight increase in the mean NR retention times measured during autumn, with values ranging between 64 (± 7.56) and 99 (± 14.01) minutes. During winter 2001, there was a slight reduction in the mean NR retention times, with values ranging between 53 (± 10.37) and 74 min (± 11.01). One-way ANOVA indicated no significant seasonal or spatial differences in the mean NRR times of *O. tigrina* ($p > 0.05$).

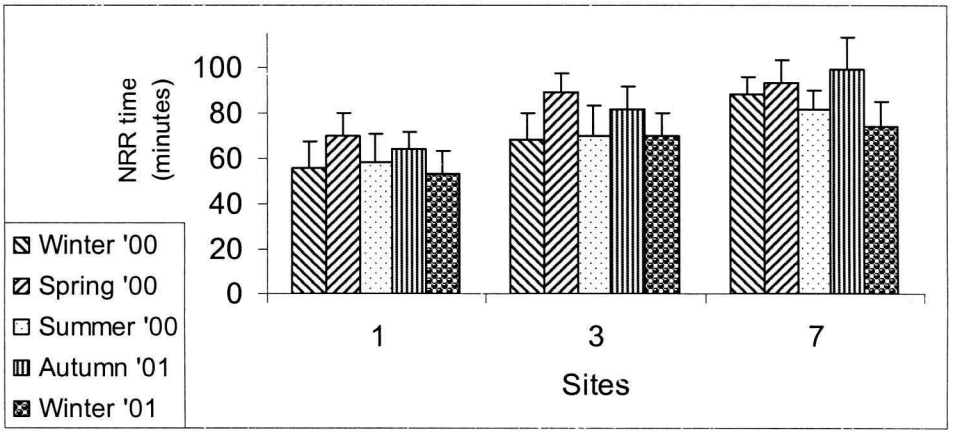

Figure 16: NRR retention times (in minutes) (mean ± SE) measured in *O. tigrina* at three sites during five seasons (Site 1- Strand; 3-Glencairn; 7-Miller's Point) (n = 5)

The NRR times measured for the mussel *C. meridionalis* are shown in Figure 17. The individuals collected during spring and autumn showed significantly longer NR retention times ($p < 0.001$) than those obtained at other times of the year (Appendix 15). The mean NRR times measured during winter 2000 ranged between 70 (± 12.94)

and 83 (± 9.19) minutes, while the spring values ranged between 110 (± 9.26) and 140 (± 12.56) minutes. During summer, the NR retention times ranged between 72 (± 9.51) and 87 (± 12.28) minutes. The autumn values ranged between 125 (± 11.15) and 136 (± 10.43) minutes, while the winter 2001 values ranged between 65 (± 15.79) and 92 (± 8.29) minutes. There were no significant differences in the mean NR retention times measured in the samples collected from the different sites ($p > 0.05$).

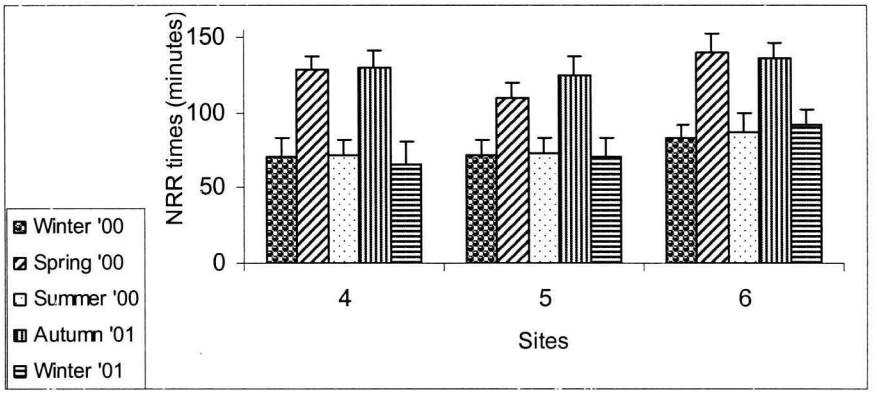

Figure 17: NRR times (in minutes) (mean ± SE) measured in *C. meridionalis* at three sites during the different seasons (Site 4-Muizenberg; 5-Rooiels; 6-Kleinmond) (n = 5)

Figure 18 shows the mean NR retention times obtained for the limpet *P. oculus*. There were significant seasonal and spatial differences in mean NRR times ($p < 0.001$). At most sites, values tended to increase during spring and autumn (Appendix 16). During winter 2000, the values ranged between 150 (± 9.19) and 187 (± 11.27) minutes, which were measured in the limpets from sites 6 and 7 respectively. The spring values ranged between 113 (± 10.18) and 200 (± 11.39) minutes, which were measured in the limpets from sites 4 and 7 respectively. During summer, the mean NRR times measured decreased to values ranging between 92 (± 7.69) and 159 (± 9.84) minutes, which were measured in the limpets from sites 1 and 7 respectively. The autumn values ranged between 133 (± 10.70) and 209 (± 12.48) minutes,

measured at sites 1 and 7 respectively, while the winter 2001 values ranged between 101 (± 8.59) and 167 (± 12.07) minutes, measured at sites 4 and 2 respectively.

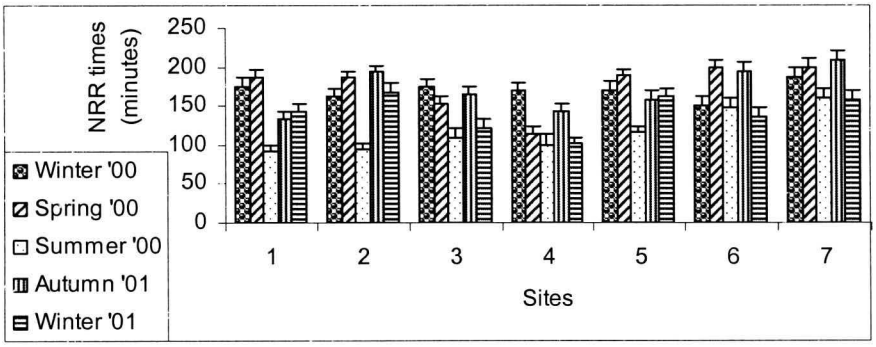

Figure 18: NRR times (in minutes) (mean ± SE) measured in *P. oculus* at seven sites during different seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point) (n = 5)

The NRR times measured in the sea star *P. exigua* are shown in Figure 19. The values obtained during winter 2000 (Appendix 17) ranged between 73 (± 7.11) and 88 min (± 8.20). During spring, the values increased slightly to values that ranged between 82 (± 5.94) and 90 min (± 9.18), decreasing again during summer to values that ranged between 71 (± 9.26) and 80 min (± 11.63). The autumn mean NR retention times increased slightly to range between 81 (± 9.26) and 87 min (± 6.62) while the winter 2001 values decreased slightly to range between 72 (± 11.60) and 82 min (± 8.68).

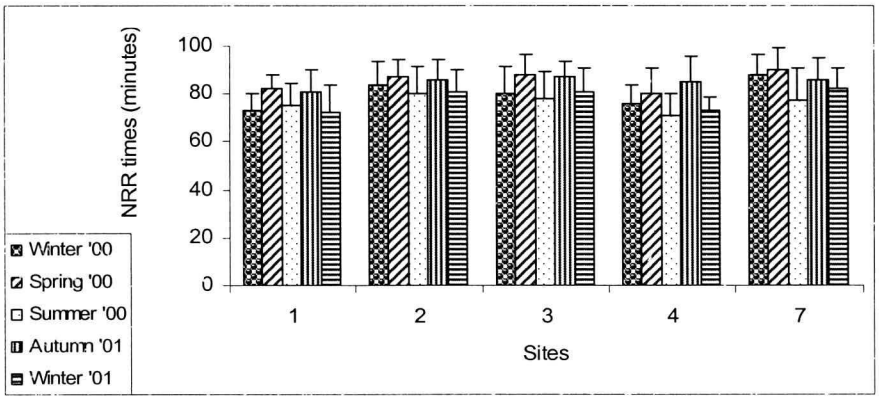

Figure 19: NRR times measured in *P. exigua* at five sites during the different seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

9.3.1.2. NRR times of laboratory-exposed animals

The mortality among the exposed organisms was observed in the group that was exposed to 400 µg/L, and was 10 % for *O. tigrina,* 17.5 % for *P. oculus*, 7.5 % for *C. meridionalis,* and 5 % for *P. exigua.* The NRR times measured during the exposure period in *O. tigrina* are shown in Figure 20. There was a gradual and progressive reduction in the mean NR retention times of all the treatment groups, although the reduction in NR retention times was slightly faster in the exposed groups. The mean NR retention times of the control group (Appendix 18) decreased from 90 (± 13.81) minutes before the start of the exposure period, to 65 (± 9.40) minutes on the last day of exposure. After the decontamination week, the NR retention time in this group increased slightly to 76 (± 9.40) minutes. In the group which was exposed to 200 µg/L, the mean NR retention times decreased from 90 (± 10.06) at the start of the exposure period, to 40 (± 14.32) minutes by the end of the exposure period, and then increased slightly to 75 (± 14.33) minutes after decontamination. In the group which was exposed to 400 µg/L, the mean NR retention times decreased from 90 (± 9.99) at the start of the exposure period, to 30 (± 11.01) minutes on day 14, and then increased slightly to 50 (± 11.01) minutes after decontamination.

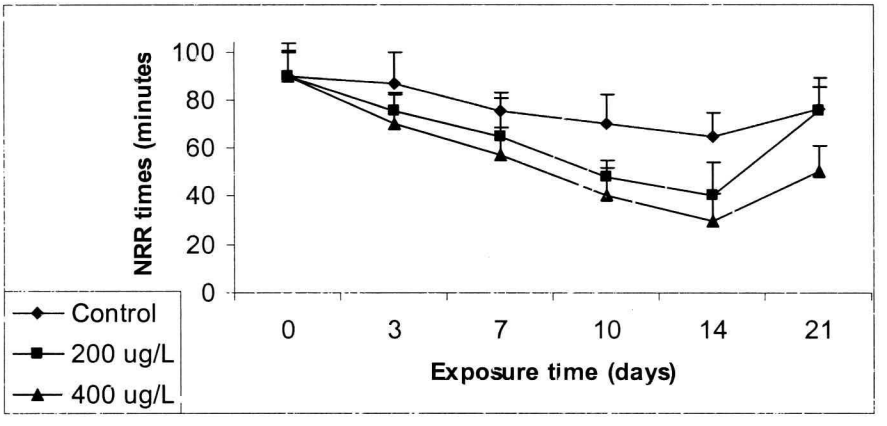

Figure 20: NRR times (in minutes) (mean ± SE) measured in *O. tigrina* during the 14-day exposure and after a week's decontamination (n = 5)

One-way ANOVA indicated significant differences ($p < 0.05$) in the mean NRR times from the 7th day until the end of the exposure period. There was, however, no significant difference between the NR retention times measured in the three groups after the decontamination week ($p > 0.05$).

The NR retention times measured in *C. meridionalis* are shown in Figure 21. There was a gradual reduction in the mean NRR times measured in all three experimental groups (Appendix 19), although the NR retention times in the control group remained significantly higher than those of the exposed groups from the 7th day until the end of the exposure ($p < 0.001$). In the control group, the mean retention times decreased from 150 (± 11.76) to 90 (± 13.95) minutes by the end of the exposure period. In the group which was exposed to 200 µg/L, the NR retention times decreased from 148 (± 8.53) to 60 (± 10.43) minutes on day 14, while in the group which was exposed to 400 µg/L, the NR retention times decreased from 148 (± 11.93) to 30 (± 11.26) minutes on the last day of exposure. After the decontamination week, the control mean NRR times increased to 110 (± 11.76) minutes, while those of the 200 µg/L and the 400 µg/L groups increased to 72 (± 8.68) and 40 (± 11.46) minutes, respectively.

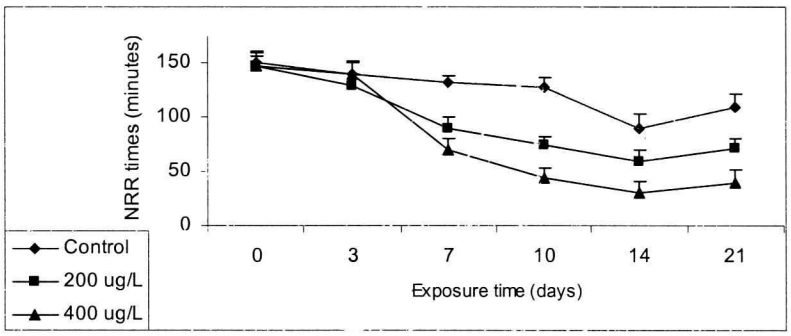

Figure 21: NRR times (in minutes) (mean ± SE) measured in *C. meridionalis* during the 14-day exposure and after a week's decontamination (n = 5)

In *P. oculus,* the NRR times of the control group (Appendix 20) decreased from 210 (± 11.76) minutes at the start of the experiment to 150 (± 12.20) minutes on day 14 (Figure 22). After decontamination, the retention time increased slightly to 170 (± 10.90) minutes. In the group which was exposed to 200 µg/L, the mean NRR times decreased from 208 (± 11.80) minutes to 105 (± 16.73) minutes at the end of the exposure period. After decontamination, the retention times increased to 120 (± 12.57) minutes. In the group which was exposed to 400 µg/L, the mean NRR times decreased from 206 (± 14.52) minutes to 75 (± 9.09) minutes, and then increased again to 90 (± 10.86) minutes at the end of the decontamination week. One-way ANOVA indicated significant differences between the mean NRR times of the control and exposed groups ($p < 0.001$) from day 7 until the end of exposure. There was also a significant difference between the mean NRR times of the control and exposed groups after the decontamination week ($p < 0.05$).

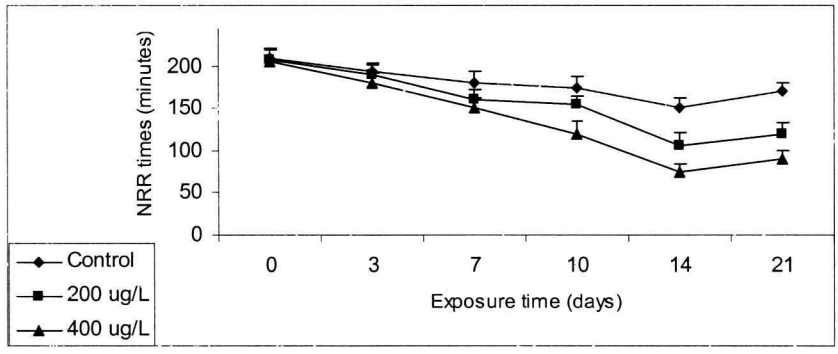

Figure 22: NRR times (in minutes) (mean ± SE) measured in *P. oculus* during the 14-day exposure and after a week's decontamination (n = 5)

For *P. exigua*, the mean NRR times of the control group (Appendix 21) ranged between 90 (± 10.14) minutes at the start of the exposure period, to 30 (± 9.94) minutes on day 14 (Figure 23). At the end of the decontamination week, the retention times increased slightly to 45 (± 9.89) minutes. The mean NRR times of the group which was exposed to 200 µg/L decreased from 89 (± 8.56) to 15 (± 9.65) minutes during the exposure period, while those of the group exposed to 400 µg/L decreased from 90 (± 12.33) minutes to 2 (± 1.30) minutes by the 14th day of the exposure

period. After decontamination, the mean NR retention time for the 200 µg/L group increased slightly to 20 (± 10.07) minutes, and to 14 (± 9.01) minutes for the 400 µg/L group. One-way ANOVA indicated significant differences between the mean NRR times of the control and those of the exposed groups from day 7 until the end of the decontamination week ($p < 0.001$).

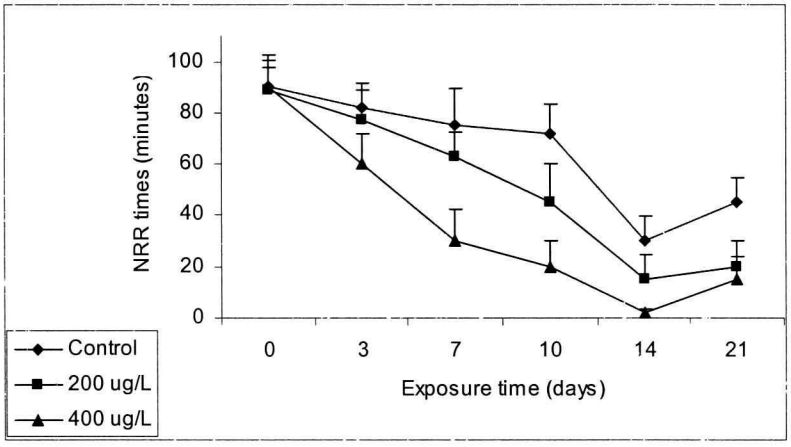

Figure 23: NRR times (in minutes) (mean ± SE) measured in *P. exigua* during the 14-day exposure and after a week's decontamination (n = 5)

9.3.2. NR lysosomal destabilization indices

9.3.2.1. Destabilization indices of field-collected animals

Figure 24 shows the lysosomal destabilization indices measured for *O. tigrina* during the five seasons. The individuals collected from site 1 showed significantly higher destabilization compared to those from the other sites ($p < 0.001$). The destabilization indices ranged between 20.5 (± 9.80) and 31.5 (± 8.06) % (Appendix 22). There were no significant seasonal differences between lysosomal destabilization indices ($p > 0.05$). The lysosomal destabilization indices of *C. meridionalis* (Appendix 23) ranged between 30 (± 10.28) and 38.9 (± 9.38) % (Figure 25). One-way ANOVA indicated no significant seasonal or spatial differences in the lysosomal destabilization indices ($p > 0.05$). Figure 26 shows the lysosomal destabilization indices of *P. oculus* measured during the different seasons at seven sites. The values ranged between 19.7 (± 9.23) and 38.6 (± 9.99) % (Appendix 24). One-way ANOVA indicated no significant seasonal differences ($p > 0.05$), while there were significant spatial

differences ($p < 0.001$) with the limpets from site 5 having higher percentage lysosomal destabilization than those of the other sites.

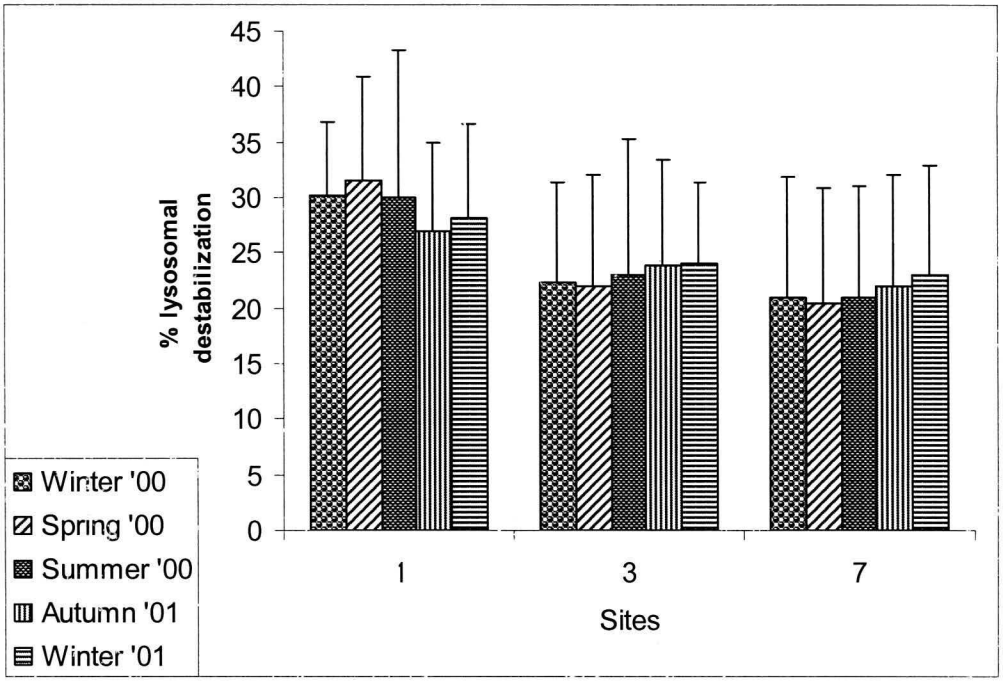

Figure 24: Lysosomal destabilization indices (mean ± SE) of *O. tigrina* collected from three sites during five seasons (Site 1-Strand; 3-Glencairn; 7-Miller's Point)

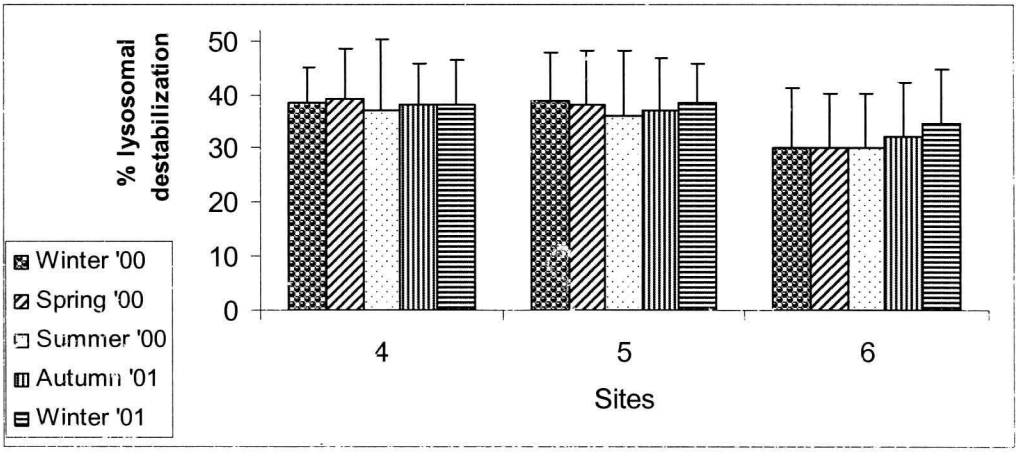

Figure 25: Lysosomal destabilization indices (mean ± SE) of *C. meridionalis* collected from three sites during five seasons (Site 4-Muizenberg; 5-Rooiels; 6-Kleinmond)

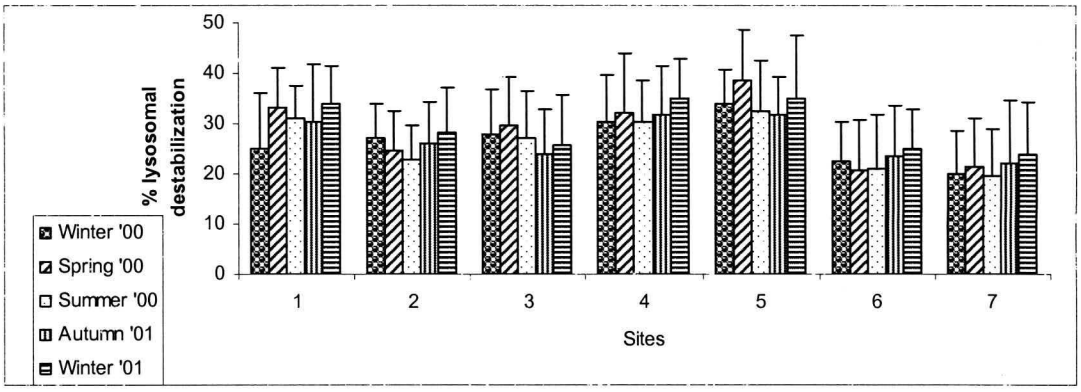

Figure 26: Lysosomal destabilization indices (mean ± SE) of *P. oculus* collected from seven sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond' 7-Miller's Point)

The lysosomal destabilization indices measured for *P. exigua* (Appendix 25) ranged between 20.2 (± 8.53) and 39.4% (± 8.03) (Figure 27). One-way ANOVA indicated significant spatial differences ($p < 0.001$), while there were no significant seasonal differences ($p > 0.05$) in the mean percentage lysosomal destabilization.

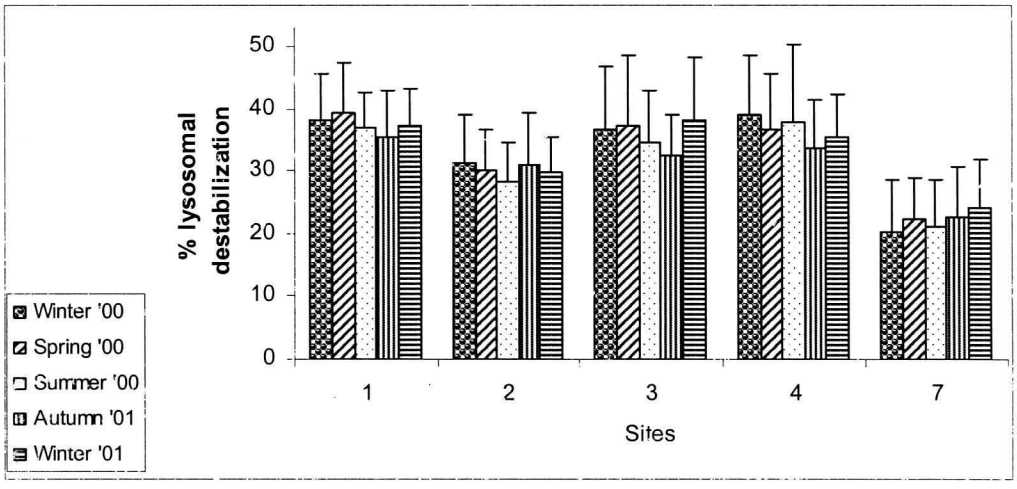

Figure 27: Lysosomal destabilization indices (mean ± SE) of *P. exigua* collected from five sites during five seasons (Site 1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 7-Miller's Point)

9.3.2.2. Destabilization indices of laboratory-exposed animals

For *O. tigrina,* the lysosomal destabilization indices (Appendix 26) of the control animals were slightly lower than those of the exposed animals throughout the exposure period, although there was a steady increase in the destabilization indices of all the groups (Figure 28). However, slight decreases in the destabilization indices of exposed individuals were observed between day 3 and day 7. The control individuals had mean destabilization indices ranging between 20.5 (± 7.55) and 26.9 (± 5.29) %, the 200 µg/L group had values ranging between 21.7 (± 7.36) and 38.4 (± 9.75) %, while the 400 µg/L group had values ranging between 20.4 (± 7.75) and 45.6 (± 11.48) %. One-way ANOVA indicated significant differences between the mean percentage lysosomal destabilization indices of the control and exposed groups from day 10 until the end of the exposure period ($p < 0.001$). After the decontamination week, the exposed groups showed some slight reduction in the lysosomal destabilization indices.

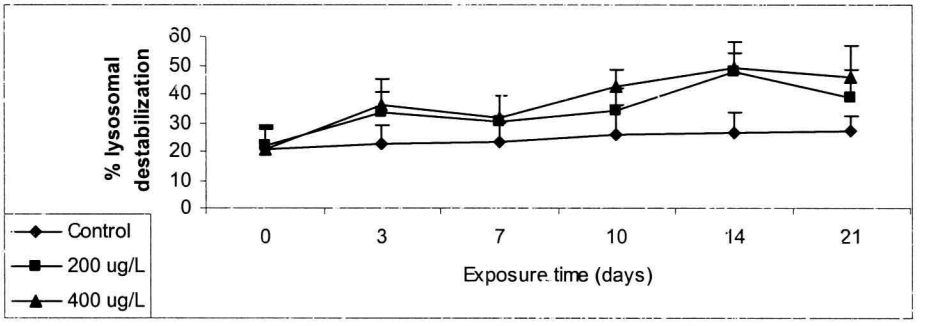

Figure 28: Lysosomal destabilization indices (mean ± SE) of the control and Cd-exposed *O. tigrina* measured during the 14-day exposure and a week's decontamination periods

The lysosomal destabilization indices measured for *C. meridionalis* are shown in Figure 29. For the control group (Appendix 27), the mean destabilization indices increased from 31.5 (± 8.01) % prior the start of the exposure period, to 37.4 (± 8.77) % on day 14. After the decontamination week, the value increased slightly to 38 (± 8.32) %. The group that was exposed to 200 µg/L had mean lysosomal destabilization indices ranging between 32.7 (± 7.51) and 57.2 (± 10.77) %, while those of the group which was exposed to 400 µg/L had destabilization indices that ranged between 30.6 (± 9.49) and 58.9 (± 8.16) %. Between days 3 and 7 of the exposure period, there were slight reductions in the lysosomal destabilization indices of both the 200 µg/L and 400 µg/L groups. One-way ANOVA indicated significant differences in the mean percentage lysosomal destabilization indices of the control and exposed groups from the 3rd day onwards ($p < 0.001$).

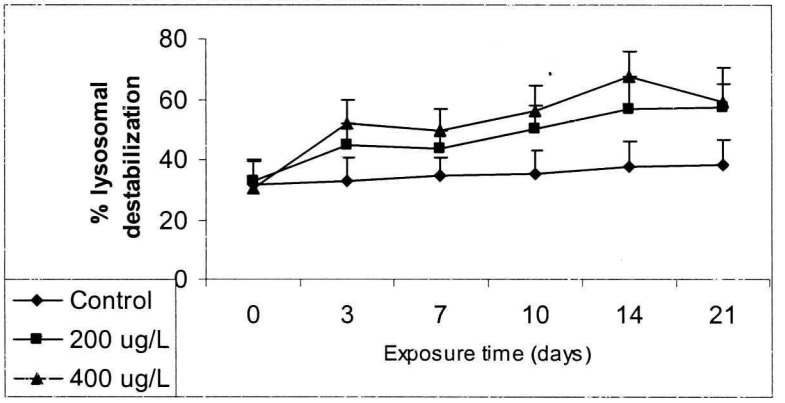

Figure 29: Lysosomal destabilization indices (mean ± SE) of the control and Cd-exposed *C. meridionalis* measured during the 14-day exposure and after a week's decontamination

The lysosomal destabilization indices of *P. oculus* are shown in Figure 30. For the control group (Appendix 28), the destabilization indices increased from 20.8 (± 8.72) % prior to the exposure period, to 27.3 (± 6.84) % at the end of the decontamination week. In the group which was exposed to 200 µg/L, the values increased from 20.7 (± 10.01) to 31.3 (± 6.89) % on day 3, decreased slightly to 28.9 (± 6.68) % on day 7, and then increased gradually for the rest of the exposure period to 58.0 (± 7.31) %. After a week's decontamination, the values decreased slightly to 50.3 (± 9.25) %. In the group which was exposed to 400 µg/L, the destabilization indices increased progressively from 24.9 (± 7.13) to 38.1 (± 9.68) % on the 3rd day, decreased slightly between day 3 and day 7 to 34.2 (± 5.71) %, and then increased gradually from day 7 onwards to reach 59.6 (± 7.93) % by the end of the decontamination period. One-way ANOVA indicated significant differences between the mean destabilization indices of the control and exposed groups from day 10 onwards ($p < 0.001$).

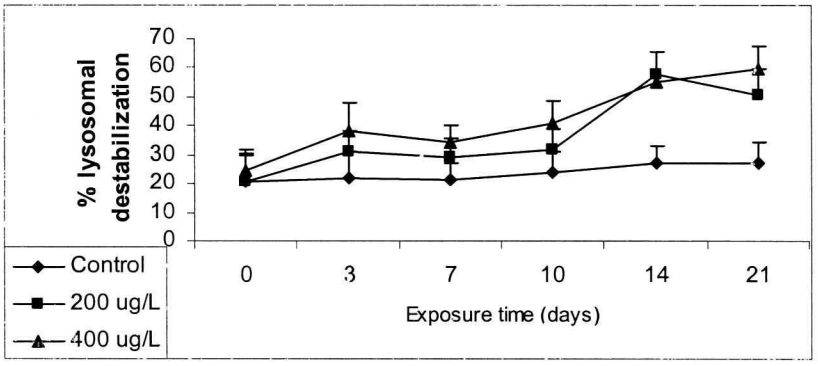

Figure 30: Lysosomal destabilization indices (mean ± SE) of control and Cd-exposed *P. oculus* measured during the 14-day exposure and after a week's decontamination

For *P. exigua,* the control group (Appendix 29) had mean lysosomal destabilization indices that increased from 40.4 (± 11.25) % at the start of the exposure, to 51.3 (± 6.88) % by day 14 (Figure 31). After the decontamination week, the destabilization indices increased slightly to 48.2 (± 6.93) %.

The 200 µg/L group had values that increased from 41.5 (± 9.75) to 66.3 (± 6.52) %, with a slight decrease to 61.2 (± 8.43) % following decontamination. In the 400 µg/L group, the destabilization indices increased from 40.9 (± 5.89) to 68.9 (± 7.36) % on day 14, decreasing slightly to 65.3 (± 8.21) % following decontamination. Between days 3 and 7, there were slight decreases in the destabilization indices of all the groups. One-way ANOVA indicated significant differences between the mean lysosomal destabilization indices of the control and exposed groups ($p < 0.001$) from day 10 until after the decontamination week.

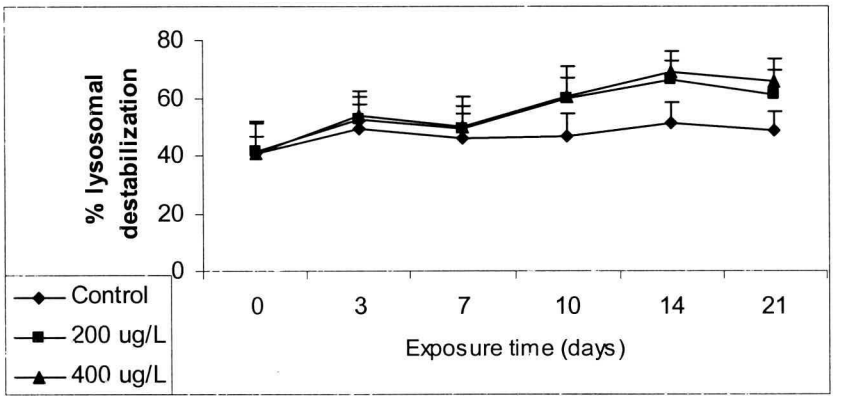

Figure 31: Lysosomal destabilization indices (mean ± SE) of control and Cd-exposed *P. exigua* measured during the 14-day exposure and after a week's decontamination

9.3.3. Viability tests

The Eosin Y exclusion test resulted in a cell viability of 85% for all haemolymph samples tested, indicating that the haemolymph extraction process and subsequent handling of organisms did not interfere excessively with cell physiology.

9.3.4. Image analysis of lysosomal size

9.3.4.1. Image analysis in field-collected animals

Table 95 shows the lysosomal sizes measured during September 2001 in the animals from the different sites. For *O. tigrina,* the mean lysosomal diameter ranged between 5.00 and 16.90 µm, with the samples from site 1 having slightly bigger lysosomes

than those from the other two sites. One-way ANOVA indicated that these inter-site differences were significant ($p < 0.05$).

TABLE 95: Lysosomal diameters (µm) (mean ± SE) measured in the different animal species from the different sites (n = 200)

O. tigrina

Sites	Mean ± SE	Range
1	11.52 ± 3.37	8.39 - 16.90
3	10 27 ± 3.38	5.27 - 13.44
7	8.69 ± 3.68	5.00 - 13.20

C. meridionalis

Sites	Mean ± SE	Range
4	12.18 ± 3.96	8.33 - 15.44
5	15.37 ± 3.80	12.84 - 20.10
6	11.32 ± 3.06	8.36 - 15.05

P. oculus

Sites	Mean ± SE	Range
1	12.71 ± 4.52	6.29 - 16.90
2	11.47 ± 3.30	6.29 - 14.82
3	8.79 ± 2.50	6.67 - 13.00
4	9.44 ± 3.98	5.98 - 15.77
5	13.66 ± 3.61	10.17 - 18.47
6	7.25 ± 2.24	6.09 - 11.24
7	9.27 ± 3.72	6.01 - 15.60

P. exigua

Sites	Mean ± SE	Range
1	16.60 ± 3.33	12.58 - 21.07
2	10.23 ± 2.67	8.39 - 14.82
3	10.64 ± 4.62	5.27 - 16.67
4	15.88 ± 2.98	11.10 - 18.79
7	11.68 ± 4.68	6.67 - 18.63

*Site1-Strand; 2-Gordon's Bay; 3-Glencairn; 4-Muizenberg; 5-Rooiels; 6-Kleinmond; 7-Miller's Point)

One-way ANOVA indicated that these inter-site differences were significant ($p < 0.05$). The mean diameters of the lysosomes of *C. meridionalis* ranged between 8.33 and 20.10 µm, with those obtained from site 5 samples being significantly larger than those of samples from the other sites ($p < 0.001$). For *P. oculus*, the mean lysosomal sizes ranged between 5.98 and 18.47 µm, with those obtained from site 5 samples

being significantly larger than those of the other samples ($p < 0.05$). The mean lysosomal diameters of *P. exigua* ranged between 5.27 and 21.07 µm, with those of samples from site 1 being significantly larger than those of samples from the other sites ($p < 0.05$).

9.3.4.2. Image analysis in Cd-exposed animals

The lysosomal sizes of all three experimental groups of *O. tigrina* (Appendix 30) increased gradually and progressively during the exposure period until the end of the decontamination week (Figures 32 & 33). For the control group, the mean lysosomal diameter increased from 8.90 (± 4.54) µm at the start of the exposure, to 15.00 (± 4.51) µm at the end of the decontamination week. For the 200 µg/L group, the mean lysosomal diameter increased from 8.93 (± 4.22) µm at the start of the exposure period, to 22.36 (± 6.56) µm at the end of the decontamination week. The mean lysosomal diameters of the 400 µg/L group increased from 8.91 (± 4.09) µm at the start of the exposure period, to 25.70 (± 5.49) µm at the end of the decontamination week. One-way ANOVA indicated significant differences between the mean lysosomal diameters of the control and the exposed groups from day 3 until the end of the decontamination week ($p < 0.001$).

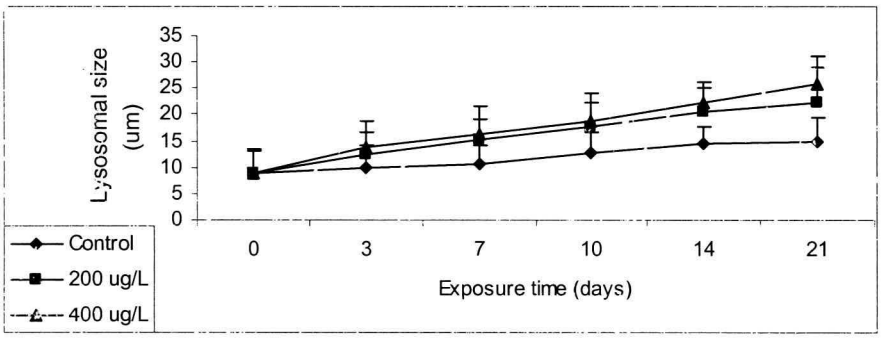

Figure 32: Lysosomal sizes (µm) (mean ± SE) of the control and Cd-exposed groups of *O. tigrina* measured during the 14-day exposure and after a week's decontamination (n = 200)

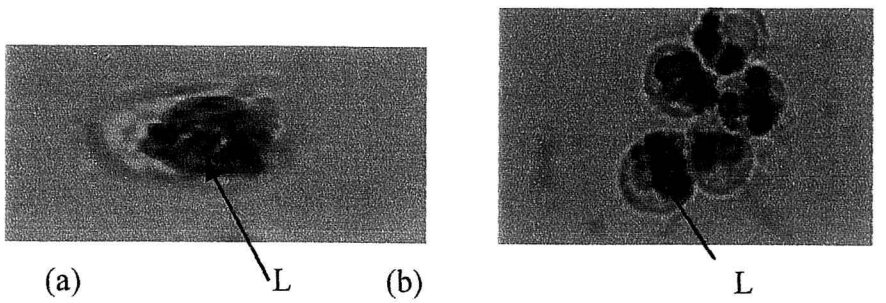

Figure 33: Micrographs showing the NR-stained lysosomes (L) of *O. tigrina* exposed to 400 µg/L prior to the exposure (a), and the enlarged lysosomes on day 14 (X100)

The mean lysosomal diameters measured in *C. meridionalis* (Appendix 31) are shown in Figure 34. Although the lysosomal sizes gradually increased in all three groups, those of the control group remained significantly smaller ($p < 0.001$) than those of the exposed groups until the end of the exposure period. The mean lysosomal diameters of the control group increased from 9.74 (± 3.83) to 16.04 (± 4.19) µm, those of the group which was exposed to 200 µg/L increased from 9.82 (± 3.73) to 25.43 (± 6.02) µm, while those of the group exposed to 400 µg/L increased from 9.75 (± 3.76) to 25.88 (± 8.18) µm. For *P. oculus,* the mean lysosomal diameters of the control group (Appendix 32) increased gradually from 6.10 (± 3.66) to 14.54 (± 4.16) µm by the end of the decontamination week (Figure 35). For the 200 µg/L group, the lysosomal sizes increased from 6.60 (± 3.97) to 19.97 (± 5.85) µm, while those of the 400 µg/L group increased from 6.58 (± 3.43) to 24.24 (± 3.58) µm. One-way ANOVA indicated that there were significant differences between the mean lysosomal sizes of the control and the exposed groups from the 7th day until the end of the decontamination week ($p < 0.001$).

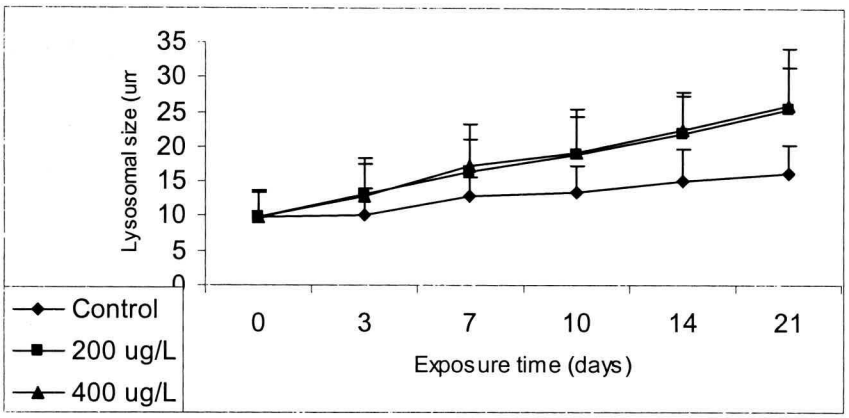

Figure 34: Lysosomal sizes (µm) (mean ± SE) of the control and Cd-exposed groups of *C. meridionalis* measured during the 14-day exposure and after a week's decontamination (n = 200)

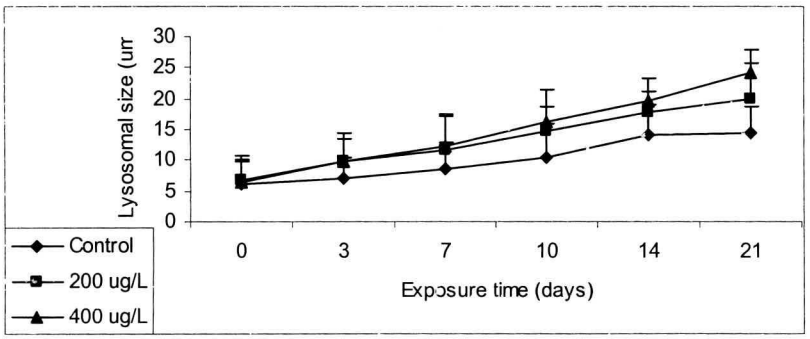

Figure 35: Lysosomal sizes (µm) (mean ± SE) of the control and Cd-exposed groups of *P. oculus* measured during the 14-day exposure and after a week's decontamination (n = 200)

Figure 36 shows the lysosomal sizes measured in the different experimental groups of *P. exigua*. The mean lysosomal diameters of the control group (Appendix 33) were significantly smaller than those of the exposed groups ($p < 0.001$) from the 7th day until the end of the decontamination week. The values for this group increased from 5.79 (± 2.94) to 14.69 (± 7.07) µm by the end of the decontamination week. For the group which was exposed to 200 µg/L. the mean lysosomal sizes increased from 5.84 (± 3.22) to 15.66 (± 4.46) µm, while those of the group which was exposed to 400

µg/L increased from 5.88 (± 2.42) to 21.34 (± 4.99) µm by the end of the decontamination week.

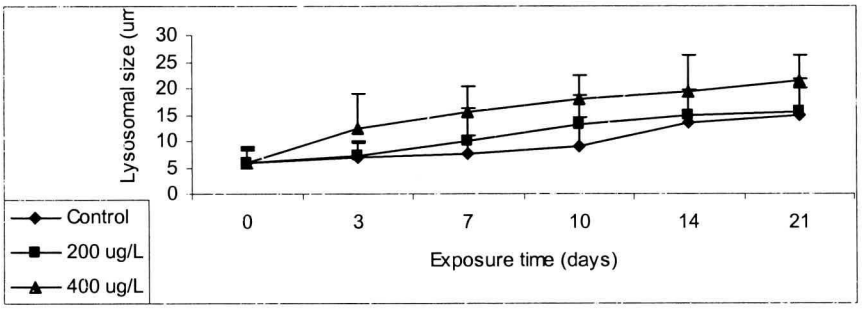

Figure 36: Lysosomal sizes (µm) (mean ± SE) of the control and Cd-exposed groups of *P. exigua* measured during the 14-day exposure and after a week's decontamination (n = 200)

9.3.5. Image analysis of lysosomal epithelial layer

9.3.5.1. Image analysis of field-collected organisms

Table 96 shows the results of the measurements of the digestive gland epithelial cell heights and the percentage epithelial areas of the different field-collected organisms. One-way ANOVA indicated significant spatial differences ($p < 0.05$) in the various parameters of the digestive gland tubules of each species. For *O. tigrina*, the samples collected from site 1 had significantly smaller mean percentage epithelial area and a thinner epithelial cell layer ($p < 0.05$). For *C. meridionalis*, the samples from site 5 had significantly smaller epithelial area and a thinner epithelial layer ($p < 0.05$). For *P oculus,* the samples from sites 1 and 4 had significantly smaller epithelial areas and thinner cell epithelial layers ($p < 0.05$).

9.3.5.2. Image analysis of Cd-exposed organisms

The percentage epithelial areas of the digestive gland tubules of three species exposed to 400 µg/L $CdCl_2$, and which were measured during the first three days of the exposure period, are shown in Table 97. The control groups had significantly higher percentage epithelial areas than the exposed organisms ($p < 0.001$). In the exposed species, the percentage epithelial areas decreased with time of exposure.

TABLE 96: Measurements (µm) of digestive gland tubule parameters in the different field-collected species

			O. tigrina		
Sites	**Tubule area** (μm^2)	**Lumen area** (μm^2)	**Epithelial area** (μm^2)	**% Epithelial area**	**Epithelial heights** (µm) **Mean** **Range**
1	9.9×10^5	3.57×10^5	6.32×10^5	63 9	42.44 (34.98- 47.32)
3	1.01×10^6	1.45×10^5	8.68×10^5	85.7	54.30 (45.81 – 58.18)
7	9.05×10^5	1.33×10^5	7.72×10^5	85.3	52.16 (49.67 – 62.89)
			C. meridionalis		
Sites	**Tubule area**	**Lumen area**	**Epithelial area**	**% Epithelial area**	**Epithelial heights** **Mean** **Range**
4	7.9×10^4	1.65×10^4	6.31×10^4	79.3	61.59 (43.71 – 778.66)
5	3.95×10^4	1.31×10^4	2.65×10^4	66.9	41.02 (37.79 – 65.80)
6	3.37×10^4	6.09×10^3	2.76×10^4	81.9	66.60 (43.71 – 78.66)
			P. oculus		
Sites	**Tubule area**	**Lumen area**	**Epithelial area**	**% Epithelial area**	**Epithelial heights** **Mean** **Range**
1	9.29×10^5	3.74×10^5	5.55×10^5	59.7	84.10 (76.70 – 98.75)
2	9.95×10^5	3.65×10^5	6.29×10^5	63.3	92.93 (84.37 – 109.18)
3	1.01×10^6	3.58×10^5	6.51×10^5	64.5	104.59 (96.28 – 118.88)
4	9.24×10^5	4.46×10^5	4.77×10^5	51.7	77.56 (64.33 – 94.79)

TABLE 97: Percentage epithelial areas (Mean ± SE) of the digestive gland tubules of organisms exposed to 400 µg/L of $CdCl_2$

Exposure group	**Species**		
	O. tigrina	***C. meridionalis***	***P. oculus***
Control	81.7 (± 0.12)	88.2 (± 0.22)	96.3 (± 0.32)
24h-exposed	63.9 (± 0.05)	69.6 (± 0.13)	78.0 (± 0.11)
48h-exposed	45.9 (± 0.21)	52.6 (± 0.15)	66.0 (± 0.33)
72h-exposed	36.8 (± 0.09)	41.6 (± 0.28)	54.7 (± 0.09)

9.3.5.3. Correlation analyses

Among the Cd-exposed groups, there was a strong negative correlation between the Cd concentrations and NRR times in all other species (r = -0.96) except *P. oculus,* which showed a weak correlation between the two variables (r = -0.78). A strong positive correlation (r = 0.96) was also observed between the Cd concentrations and the destabilization indices in the Cd-exposed groups in all other species except the *O. tigrina* which were exposed to 0.2 mg/L $CdCl_2$, which showed a weak positive correlation between the two variables (r = 0.77). There was also a strong positive correlation (r = 0.98) between the Cd concentrations and the lysosomal sizes of the Cd-exposed individuals.

9.4. DISCUSSION

The results of the present study showed significantly longer NRR times during spring and autumn in the field-collected *C. meridionalis* and *P. oculus,* while there was a reduction in the NRR times measured during summer and winter (Figures 17 & 18). A reduced NRR time is, according to Svendsen & Weeks (1994), an indication of higher stress levels, which may mean that the field-collected organisms in the present study were under more stress during winter and summer. Site-related differences in the NRR times were found only in the samples of *P. oculus* (Figure 18), with the longer NRR times in the site 7 specimens possibly indicating lower contamination levels at this site. According to Dailianis et al. (2003), lysosomal membrane destabilization is caused by both natural and cytotoxic stressors, thus it may be possible that the transportation of the field-collected animals in 5-L plastic buckets was, in itself, a stress-inducing situation that may have contributed to the lysosomal membrane destabilization observed. Starvation could also have had similar effects, as seen in the slight reduction in NRR times of control organisms (Figures 20-23).

Although no significant spatial differences in the NRR times of the other species besides *P. oculus* were observed, there were significant differences in the destabilization indices of *O. tigrina* (Figure 24), *P. oculus* (Figure 25) and *P. exigua* (Figure 27). The higher percentage lysosomal membrane destabilization which was observed in the field-collected organisms from site 1 may be indicative of the

contaminated conditions at this site resulting from the presence of a number of stormwater outlets at this site (Taljaard et al, 2000). The lysosomal membrane destabilization of the organisms collected from site 5 may be related to the historic pollution inputs from the military weapons testing activities suggested earlier (Chapter 2).

The reduction in the NRR times of the Cd-exposed organisms is in agreement with other findings (Lowe et al., 1995; Nicholson, 1999b). In the laboratory study reported on here, the control groups retained the NR dye for significantly longer periods than the groups exposed to $CdCl_2$ did (Figures 20 – 23), indicating that the lysosomes of the exposed groups were one of the targets of contaminants, as suggested previously by Lowe & Pipe (1994). The slight increases in the NRR times of exposed groups after decontamination may be evidence of an improvement in the lysosomal membrane stability according to Viarengo et al. (2000) in a study they did on mussels. Although environmental stressors such as starvation could have caused the decrease in NRR times of the control organisms (Figures 20-23), the control values were maintained at fairly stable and higher levels compared to the exposed groups.

The higher percentage destabilization in the Cd-exposed organisms may be an indication of lysosomal dysfunction caused by contaminant exposure (Regoli, 1992). The slight decrease observed in the destabilization indices between days 3 and 7 of the exposure period may be due to compensatory mechanisms found in contaminated organisms in response to stress (Ringwood et al., 1998). This time interval was found to be typical of the delay associated with the induction of metallotheioneins (MTs) for the sequestration of Cd in bivalves (Ringwood et al., 1998).

The image analysis of the haemolymph samples of organisms collected from sites 1 and 5 showed significantly enlarged lysosomes (Table 95) which may be related to the general lysosomal destabilization caused by the increased contamination levels at these sites (Chapter 2). This is in agreement with the findings of Etxeberria et al., (1995) that larger lysosomes were associated with animals collected from areas with poor water quality.

The extent of lysosomal enlargement and depressed lysosomal membrane stability observed in the Cd-exposed animals (from the laboratory study) (Figures 32 – 35) was higher than that observed in the field-collected animals (Table 95). This is in agreement with previous findings of significant increases in lysosomal diameters of mussels following Cd loading (Sternlieb & Goldfisher, 1976). The lack of a reduction in the lysosomal sizes of the Cd-exposed groups after a week of decontamination in the present study (Figures 32- 35) may be an indication that this period was insufficient time for recovery of lysosomal size, as was found by Regoli (1992) in whose study significant reduction in lysosomal size was found only after 12 days' decontamination.

The reduction in the percentage epithelial areas seen in the digestive gland samples of *O. tigrina* from site 1, *C. meridionalis* from site 5, and *P. oculus* from sites 1 and 4 (Table 96) may be a reflection of the presence of contamination, probably due to the sewage effluent and road runoff being discharged at these sites (Taljaard et al., 2000). The reduction in the percentage epithelial areas of the Cd-exposed groups was evident within 24 hours of exposure (Table 97), indicating that contaminant exposure effects can be detected readily. This response may be an indication of the digestive gland epithelial atrophy due to the degenerative effects of contaminant exposure (Couch, 1984). According to Snyman (2001), the reduction in the digestive gland epithelia and an increase in lumen size may be due to the loss of apical cytoplasm involved in the detoxification process.

9.5. CONCLUSION

The results of this part of the study indicated that the haemolymph of aquatic invertebrates may be the target of environmental contamination, and that the haemolymph lysosomes exhibited structural changes. The lysosomes of toxically stressed organisms retained the neutral red dye for shorter periods than those of unstressed organisms. Some amount of recovery from exposure effects were seen in the slight increase of retention times after decontamination. The results also indicated that some compensatory mechanism may be activated which could reduce adverse

effects. The image analysis showed an increase in lysosomal sizes and a reduction in the digestive gland tubule epithelia of exposed organisms, which is a further indication of cellular disturbance. It may be concluded that the lysosomal stress response is a rapid response that can be measured quantitatively and can be used as a general biomarker of toxic stress caused by environmental contamination and heavy metal exposure.

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CHAPTER 10- SYNTHESIS AND GENERAL CONCLUSION

The results of the present study show that none of the catchments draining into the catchment area have escaped the impacts of anthropogenic contamination, with most of the heavy metal contamination seemingly associated with the northern shores between Strand (site 1) and Muizenberg (site 4), where the most populated and industrialised areas occur. The occasionally elevated metal concentrations that were observed at Gordon's Bay (site 2), especially for Pb during summer, may be related to the sheltered nature of this area (Taljaard et al. 2000) which may thus make it vulnerable to the accumulation of current-borne contaminants. The situation at Gordon's Bay may further be influenced by the complex circulation patterns that develop in the area under the south-easterly winds (Atkins, 1970). The Pb contamination at this site was probably linked to the presence of a yachting harbour and a boat launching pad. Fuel and oil spills from the boats in the yachting harbour and launching pad, which may have spilled onto the tarmac, may have been washed away by rain as road runoff, resulting in the accumulation of Pb in the sediments at this site (Taljaard et al, 2000). According to EPA (2003), roads, highways and bridges are sources of significant contribution of pollutants to aquatic environments, with runoff-related pollution being associated with rain that washes off these impermeable surfaces. Although many countries, including South Africa, have significantly reduced the lead levels in the environment by introducing lead-free fuel, anthropogenic lead is still widely used as an additive of gasoline and is still evident in elevated levels in urban areas (WRI, 1999).

It appears that the pollution of the False Bay intertidal zone may also be related to the various land uses that have taken place in the past, such as the weapons testing facility in the catchment, draining at Rooiels (site 5), and to those that continue to occur at present, including agricultural, industrial, fishing and boating, residential developments, holiday and recreational activities, solid waste dumping and wastewater treatment. The situation observed at Rooiels (site 5) is an example of how unsustainable developmental activities can impact on the environment, which is a trend that sustainable development is hoping to bring to a halt. The different land use patterns may contribute to the contamination of False Bay in varying degrees, with the situation being further complicated by the seasonality and bi-directional wind regime

that occurs, resulting in widely varying heavy metal concentrations both seasonally and spatially.

The heavy metal assessment in the present study has revealed a strong seasonality in pollutant inputs, which seems to be influenced by a number of factors. However, water salinity and pH seemed to be important factors in the seasonal variations of heavy metal concentrations, especially at Strand (site 1), Rooiels (site 5) and Kleinmond (site 6), probably due to the influence of freshwater inflows at the Lourens, Rooiels and Kleinmond River mouths which open at these sites respectively. In the present study, the seasonal maxima coincided with the period of greatest runoff during winter, an indication that heavy metal availability from land-based sources may be greater at this time, and is a definite source of heavy metal contamination in the intertidal zone of the bay. Globally, sewage remains the largest source of contamination of marine and coastal environments (GESAMP, 2001a). The toxicity of urban stormwater systems is well recognised (Rossi et al., 2003), and is attributed to diffuse pollution sources. The presence of a number of stormwater and sewage outlets which discharge directly into False Bay at most of the sampling sites may have contributed to the heavy metal contamination via road runoff and stormwater effluent.

Over the duration of the present study, there was evidence that the sediments accumulated contaminants, and the presence of elevated heavy metal concentrations in the sediments from Rooiels (site 5) was an example of how sediments can act as sinks of heavy metals. Although the military weapons testing activities that took place at this site had been decommissioned in the early 1990s (Cock & McKenzie, 1998), residual heavy metals may still persist, as indicated by the concentrations of Pb and Cd which probably become mobilised by rainfall from time to time. Although it is difficult to answer the question of what constitutes potentially harmful levels of contaminants in the marine environment, it is clear that the monitoring of both the land-based and marine-based sources of pollution is vital for the protection of the coastal environment. Analyses of the sediments may thus provide valuable information on the historical inputs of contaminants in the study area, and can be used

to assess threats to biota over time. Although water quality guidelines are in place in South Africa, they may have evolved within a limited ecotoxicological framework which only considered ambient concentrations without taking into account biological effects. Further research into the historic pollution inputs at the different sites of the study area can be carried out by using vertical sediment profiles, which will then provide a record of the depositional history of heavy metals in the False Bay intertidal zone.

The lack of temporal trend information for the heavy metals studied in False Bay is a significant knowledge gap. There is a need to establish whether contamination inputs in the study area are increasing or not. There is also no information on the contamination levels in marine fish and mammals from the bay. Contamination data from the marine food web is important because it leads to an understanding of the pathways of bioaccumulation of contaminants (Muir et al., 1999). No specific studies have been carried out on large mammals to determine these issues regarding False Bay species. Thus, it would be of interest to determine whether other marine animals frequenting False Bay waters have elevated heavy metal levels or not.

The heavy metal concentrations in the water varied greatly over time and with locality, which may indicate the influence of tidal cycles, freshwater runoff and seasonality. The rationale behind the present study was that an analysis of heavy metal levels in a range of different species from False Bay may give a broad picture of how the different species accumulate heavy metals. The results of this study, therefore, provide a snapshot of metal levels in selected invertebrates, and the partitioning of metals between the soft tissues and shells, where applicable. Generally, higher heavy metal concentrations in either the water or the sediment compartment corresponded to elevated levels in the field-collected biota. The body concentrations of the heavy metals were often much higher than those of the surroundings, and varied greatly between species, especially for Cd, Pb and Zn. The results of the present study revealed that even closely related species, such as *O. tigrina* and *O. sinensis,* might exhibit distinct accumulation strategies for heavy metals, resulting in widely differing concentrations of any given metal in the different organs. Although the natural

variations within species tended to obscure trends in metal accumulation, clear temporal trends were identified in the present study, and indicated important differences between species with respect to biomonitoring capacity.

A comparison of the metal levels between the species under investigation shows that the barnacle *Tetraclita serrata* accumulated Cd and Zn to a larger extent than the other organisms, and may thus be regarded as suitable biomonitors for these heavy metals. The barnacle samples collected from site 5 (Rooiels) may have reflected integrated heavy metal concentrations over time. Since the barnacles were also readily available at all the sample sites and throughout the coast of False Bay, they may, as filter feeders, serve as a possible alternative to mussels as biomonitors of heavy metals in False Bay. The high concentrations of Cu measured in *O. tigrina* seemed to be related to the presence of the copper-containing respiratory pigment haemocyanin (Rao et al., 1985), which is abundant in the haemolymph of gastropods, rather than to local inputs. Thus, *Oxystele* species cannot be regarded as suitable biomonitors of Cu, therefore making it necessary that other biomonitors besides *Oxystele* be identified for the monitoring of Cu contamination in False Bay. The two species, however, seemed to be suitable biomonitors of Cd and Zn. The accumulation of heavy metals in the shell compartment of the bodies of *Oxystele* made the shells valuable indicators of heavy metal contamination and pointed to the potential usefulness of shell concentrations in long-term biomonitoring programmes. Generally, the heavy metal uptake and accumulation levels in gastropods seemed to be less than in the filter-feeding mussels and barnacles, presumably because of the lower amount of ventilation and ingestion rates in gastropods (Wang & Ke, 2002).

In the mussel *C. meridionalis*, seasonal maxima of heavy metal concentrations coincided with increases in environmental levels. This can probably be linked to their filter feeding habit during which large volumes of water are taken in, and the high proportion of permeable surfaces. In the present study, *C. meridionalis* was mostly associated with sand at the sites where it was found, which probably exposed the mussels to the elevated heavy metal concentrations of the sediment milieu. This would explain the accumulation of maximum levels of Cd, Pb and Zn into the soft tissues of the specimens from Rooiels (site 5). As sessile organisms, the mussels

would have accumulated the heavy metals over time, and were probably the most relevant and suitable biomonitors to reflect the impacts of the historic contamination. It is difficult to compare the results of metal concentrations reported from other marine environments with the results obtained for the False Bay species in the present study, due to the lack of published data of biomonitoring studies using the same species elsewhere.

The use of biomonitors is advantageous in the case of heavy metal contamination, especially Cd, which is often below detection limits in the water and sediments. In the present study, both the soft tissues and shells of the different organisms were found to accumulate heavy metals, with the data revealing that soft tissues and shells accumulate bioavailable metals in very different ways. These results could form a basis for using shells as biomonitoring materials of heavy metals. According to Cravo et al. (2003), shells have practical advantage over soft tissues with regards to monitoring metal contamination, since they show less variability and are not influenced by changes in the physiological condition of the organism. Generally, soft tissue metal concentrations show greater variability than shells, due to the seasonal weight changes associated with the physical condition and reproductive state of the organism, thus shells may provide a more realistic indication of the degree of contamination. What is lacking, however, and requires further research, is the knowledge of metal-binding characteristics that are responsible for the accumulation of metals such as Cd and Pb at higher levels in the shells than in the soft tissues.

There is evidence from previous studies (Al-Thaqafi & White, 1991) that the position of the animals on the shore, and thus the degree of the wave action to which they are exposed, may influence metal concentration. Further research is thus needed in this regard concerning the False Bay species. Previous studies showed that there was increased metal uptake by aquatic organisms at reduced salinity (Blackmore & Wang, 2002), which has been attributed to the increased free metal ion concentrations as chloride complexation decreases. According to Leung et al. (2002), the water salinity in intertidal zones can fluctuate on hourly, daily, weekly or monthly time scales, and

these changes can affect metal bioavailability. The pH variability observed in the present study cannot be explained, although other authors (Philp, 1999) have related it to runoff events following heavy rains. According to Rensing & Maier (2003), even small changes in pH can decrease metal solubility and thus its bioavailability by several orders of magnitude. It may be assumed, therefore, that the variations in pH which were observed in the present study also contributed to the variations in heavy metal uptake and consequently, body concentrations.

In the past, resource managers have relied on chemical toxicity tests and water chemistry data to determine the quality of surface waters (Butcher et al., 2003). Recently, a resurgence in the use of biomarkers to monitor and assess ecosystems has occurred, due to a recognition of their ability to integrate effects of chemicals, which can be useful in assessing the status of polluted ecosystems (Svendsen et al., 2003). A criticism that has been levelled against biomarkers is their lack of specificity in most cases, being induced by more than one pollutant and a range of natural environmental stressors (Depledge et al., 1995). However, extending the biomarker concept from purely biochemical measurements to include measures of cellular and behavioural pathology creates the possibility of using a hierarchy of biomarkers. Thus, pollutants might initially be detected by non-specific biomarkers such as the lysosomal membrane stability biomarker, at sites which are at risk from pollution, leading to measurements of more specific biomarkers, in order to gain an overall assessment of pollutant impact (Depledge et al., 1995). A research challenge for biomarker application arising from the present study is to relate biomarker responses to heavy metal contamination in False Bay to effects at population level. This will allow for predictions of future impacts.

Cellular responses to contaminant exposure provide the link between molecular events and those occurring at more complex biological levels (Marigomez & Baybay-Villacorta, 2003). One characteristic pathological alteration that occurs in stressed organisms is decreased integrity of lysosomal membranes. According to Dailianis et al. (2003), lysosomal stability constitutes a useful index of cellular damage. The results of the exposure experiments indicated that, at cellular level, lysosomes were the targets of contaminants, and that the lysosomal membrane integrity was

compromised following Cd exposure. A clear dose-response relationship between neutral red retention times and Cd exposure was seen. The enlarged lysosomes in Cd-exposed organisms were indicative of the lysosomal membrane destabilization that accompanies contaminant exposure and toxic stress. According to Nicholson (2003), the sequestration of heavy metals within the lysosomes of marine organisms appears to alter the membrane permeability. The evidence of lysosomal destabilization effects within 24h of exposure indicated that cellular effects could be detected readily and may serve as early warning signals to environmental perturbations. The challenge is to identify the levels of lysosomal destabilization than can be sustained before adverse effects on important biological and physiological activities such as growth and reproduction are seen, or when the conditions reach the "point of no return".

The question of persistence of a biomarker response involves both transience and reversibility (Svendsen et al., 2003). The results of the present study also revealed that the damage to lysosomes did not appear to be transient, and suggest that some recovery of lysosomal membrane integrity was possible if the animal's threshold tolerance levels were not overstepped and its adaptive mechanisms were still intact. According to Svendsen et al. (2003), the potential for recovery of neutral red retention times is dependent on the production of new coelomocytes. These authors have previously suggested that a decrease in NRR time to less than 15 minutes will be linked to adverse effects of some life parameters in earthworms. There is a need therefore, for further research into the threshold NRR times which would accompany observable effects on life-cycle parameters of the different False Bay species.

The changes that were observed in the lysosomal system demonstrates their potential usefulness as sensitive biomarkers of contaminant exposure and water quality in coastal environments. A limitation of the present study is the fact that the evaluation of lysosomal structural parameters (membrane stability and size) were conducted by measuring all lysosome types without differentiating between secondary lysosomes and residual bodies, as was done by Domouhtsidou & Dimitriadis (2001). This was not feasible because of the complexity in the ultrastructural characteristics of these organelles. Further research needs to be done in the identification of the different types of lysosomes in these species.

Since the laboratory experimental exposures in the present study were carried out in a static-flow system, caution must be exercised in extrapolating these results to field conditions which would be more suitably represented by a flow-through system. Comparisons of the contaminant levels in species occurring in the wild to those of effects based on laboratory tests have inherent weaknesses. Laboratory organisms are most often exposed to single toxicants for relatively short periods of time, while natural populations are exposed to mixtures of toxicants over most of their lifetime (Beeby, 2001). Care must be taken, therefore, when interpreting the results of the exposure experiments which investigated the lysosomal effects of a single metal, that is, cadmium, because of the fact that in natural systems, groups of metals often act synergistically by having additive effects on the health of organisms (Honkoop et al., 2003). Perhaps the biggest challenge to our ability to measure and evaluate the effects of metals are metal mixtures, because the combinations that occur in nature are endless (Peakall & Burger, 2003). Thus, it would be of interest to evaluate the synergistic effects of the metals studied in the present work on the lysosomal membrane stability and lysosomal size in the different False Bay species, in order to make predictions in the field.

The state of knowledge of the dynamics of the five heavy metals studied in False Bay in the present study is inadequate for predictions to be made on the future trends of bioaccumulation from water or food in the species that occupy top positions in marine food chains, including humans. A future goal would be to use the present results along with future studies in the prediction of spatial and temporal trends in the contamination levels in False Bay top predators such as fish and mammals, in order to make the linkages of food chain models for contaminant accumulation by marine biota to models of human dietary exposure and biological effects in top predators.

For South Africa, where the economy is heavily dependent on the country's natural resources and biodiversity, threats from pollutants such as heavy metals may impact negatively on such economic activities. The biomarker responses identified in the present study can prove to be useful to the process of Integrated Coastal Management by providing early warning of pollution and thus alerting resource managers to potential contamination threats, thereby allowing them to halt or mitigate the impacts

before critical coastal and marine resources are degraded. Integrated Coastal Management seeks to bring together all those involved in the development, management and use of coastal resources, and those whose activities affect these resources within a framework that facilitates cohesion of interests and responsibilities, and encourages sustainable development. Thus, robust indicators of ecosystem health are required to help resource managers to minimise adverse impacts. Unlike physical parameters which may be useful only during an impact, biomarkers are accumulative and may be observable even after the event that caused them has passed (Linton & Warner, 2003). Thus, episodic pollution events and low-intensity, chronic impacts which may be undetectable by physical-chemical measures, may be easily detectable using the biomarker approach. In conclusion, biomarkers, as indicators of ecosystem health, can inform resource managers and policy makers about the effectiveness of the strategies put in place towards achieving sustainability. They can also be used to monitor the conditions of coastal environments on an ongoing basis to compare conditions at different localities, and measure performance and results of pollution-monitoring policies or actions.

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APPENDICES

Appendix 1: Mean Cd concentrations (± SE) (µg/g) measured in the body samples of the control group of *O. tigrina* during the 14-day exposure (n = 5)

Exposure time(days)	Soft	Shell	Gland	Foot
0	6.45 ±0.15	2.33 ± 0.10	23.10 ± 0.14	14.84 ± 0.10
3	5.46 ± 0.10	2.03 ± 0.10	22.90 ± 0.10	13.33 ± 0.10
7	4.38 ± 0.10	2.00 ± 0.14	21.40 ± 0.10	12.14 ± 0.12
10	4.25 ± 0.11	1.90 ± 0.13	20.70 ± 0.11	11.37 ± 0.10
14	4.10 ± 0.12	1.45 ± 0.16	19.45 ± 0.14	10.22 ± 0.12

Appendix 2: Mean Cd concentrations (± SE) (µg/g) measured in the body samples of *O. tigrina* exposed to 200 µg/L during the 14-day exposure and after 1 week's decontamination (n = 5)

Exposure time (days)	Soft	Shell	Gland	Foot
0	7.38 ± 0.10	2.05 ± 0.12	22.90 ± 0.12	16.00 ± 0.11
3	15.60 ± 0.11	5.00 ± 0.12	24.37 ± 0.10	19.23 ± 0.11
7	45.60 ± 0.10	6.20 ± 0.12	29.63 ± 0.10	22.22 ± 0.10
10	47.58 ± 0.13	7.35 ± 0.12	44.44 ± 0.10	27.63 ± 0.11
14	64.00 ± 0.14	9.15 ± 0.20	48.28 ± 0.10	34.0 ± 0.13
21	33.40 ± 0.11	3.07 ± 0.10	35.30 ± 0.15	27.35 ± 0.10

Appendix 3: Mean Cd concentrations (± SE) (µg/g) measured in the body samples of *O. tigrina* exposed to 400 µg/L during the 14-day exposure and after 1 week's decontamination (n = 5)

Exposure time (days)	Soft	Shell	Gland	Foot
0	10.55 ± 0.13	1.03 ± 0.10	27.25 ± 0.13	13.80 ± 0.12
3	54.00 ± 0.11	5.67 ± 0.14	42.86 ± 0.10	14.29 ± 0.10
7	69.60 ± 0.12	9.20 ± 0.10	43.48 ± 0.11	24.82 ± 0.10
10	85.60 ± 0.12	17.80 ± 0.12	57.14 ± 0.10	39.82 ± 0.10
14	122.00 ± 0.13	31.00 ± 0.12	72.00 ± 0.12	63.53 ± 0.15
21	110.09 ± 0.16	27.00 ± 0.12	63.48 ± 0.12	50.97 ± 0.10

Appendix 4: Mean Cd concentrations (± SE) (µg/g) measured in the body samples of the control group of *C. meridionalis* during the 14-day exposure (n = 5)

Exposure time(days)	Soft	Shell	Gland	Gills	Kidney
0	9.60 ± 0.10	7.41 ± 0.11	10.00 ± 0.11	0.90 ± 0.12	10.50 ± 0.10
3	9.00 ± 0.14	6.33 ± 0.10	9.55 ± 0.11	0.80 ± 0.11	7.78 ± 0.11
7	8.05 ± 0.14	5.00 ± 0.19	7.44 ± 0.10	0.60 ± 0.12	6.20 ± 0.12
10	7.13 ± 0.10	4.87 ± 0.11	6.30 ± 0.10	0.20 ± 0.12	5.20 ± 0.11
14	6.30 ± 0.10	3.99 ± 0.12	5.20 ± 0.10	ND	5.07 ± 0.17

Appendix 5: Mean Cd concentrations (± SE) (µg/g) measured in the body samples of *C. meridionalis* exposed to 200 µg/L during the 14-day exposure and after 1 week's decontamination (n = 5)

Exposure time(days)	Soft	Shell	Gland	Gills	Kidney
0	5.40 ± 0.16	2.33 ± 0.10	8.70 ± 0.10	8.00 ± 0.16	10.00 ± 0.18
3	7.00 ± 0.19	4.59 ± 0.12	12.50 ± 0.10	10.77 ± 0.14	17.90 ± 0.10
7	21.00 ± 0.16	10.00 ± 0.13	18.30 ± 0.12	15.70 ± 0.14	35.00 ± 0.17
10	34.00 ± 0.21	20.80 ± 0.12	21.74 ± 0.17	27.98 ± 0.10	45.71 ± 0.11
14	69.50 ± 0.10	30.00 ± 0.13	31.84 ± 0.10	30.00 ± 0.13	60.00 ± 0.15
21	38.70 ± 0.12	15.69 ± 0.16	19.70 ± 0.13	4.70 ± 0.13	29.80 ± 0.12

Appendix 6: Mean Cd concentrations (± SE) (µg/g) measured in the body samples of *C. meridionalis* exposed to 400 µg/L during the 14-day exposure and after 1 week's decontamination (n = 5)

Exposure time(days)	Soft	Shell	Gland	Gills	Kidney
0	10.00 ± 0.16	8.70 ± 0.11	12.50 ± 0.12	10.00 ± 0.15	12.00 ± 0.12
3	21.00 ± 0.16	10.50 ± 0.31	16.70 ± 0.14	15.39 ± 0.10	15.20 ± 0.11
7	149.00 ± 0.19	40.00 ± 0.24	23.55 ± 0.15	20.55 ± 0.14	43.90 ± 0.17
10	162. 0 ± 0.13	52.80 ± 0.11	34.78 ± 0.14	40.00 ± 0.19	67.40 ± 0.11
14	197.00 ± 0.41	80.00 ± 0.13	52.17 ± 0.13	60.00 ± 0.12	80.00 ± 0.15
21	169.70 ± 0.12	55.60 ± 0.17	33.69 ± 0.11	40.06 ± 0.20	67.79 ± 0.13

Appendix 7: Mean Cd concentrations (± SE) (µg/g) measured in the body samples of the control group of *P. oculus* during the 14-day exposure (n = 5)

Exposure time (days)	Soft	Shell
0	20.08 ± 0.15	2.18 ± 0.15
3	19.83 ± 0.10	1.25 ± 0.10
7	18.77 ± 0.16	0.99 ± 0.14
10	16.67 ± 0.13	0.67 ± 0.13
14	14.38 ± 0.11	0.41 ± 0.11

Appendix 8: Mean Cd concentrations (± SE) (µg/g) measured in the body samples of *P. oculus* exposed to 200 µg/L during the 14-day exposure and after 1 week's decontamination (n = 5)

Exposure time (days)	Soft	Shell
0	19.60 ± 0.12	2.67 ± 0.14
3	25.00 ± 0.19	13.00 ± 0.11
7	32.00 ± 0.15	15.60 ± 0.16
10	76.00 ± 0.17	25.80 ± 0.10
14	110.00 ± 0.13	51.67 ± 0.12
21	65.77 ± 0.12	32.19 ± 0.14

Appendix 9: Mean Cd concentrations (± SE) (µg/g) measured in the body samples of *P. oculus* exposed to 400 µg/L during the 14-day exposure and after 1 week's decontamination (n = 5)

Exposure time (days)	Soft	Shell
0	20.22 ± 0.11	3.07 ± 0.10
3	27.27 ± 0.16	16.00 ± 0.12
7	42.00 ± 0.12	18.70 ± 0.17
10	136.00 ± 0.18	41.80 ± 0.12
14	140.00 ± 0.19	69.90 ± 0.21
21	114.63 ± 0.10	55.40 ± 0.12

Appendix 10: Mean Cd concentrations (± SE) (µg/g) measured in the body samples of the three experimental groups of *P. exigua* during the 14-day exposure and after 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	5.25 ± 0.10	5.07 ± 0.14	5.13 ± 0.12
3	5.00 ± 0.13	5.25 ± 0.15	5.29 ± 0.13
7	4.50 ± 0.10	6.52 ± 0.10	7.50 ± 0.13
10	4.00 ± 0.13	7.25 ± 0.10	9.20 ± 0.10
14	3.85 ± 0.15	9.00 ± 0.20	11.50 ± 0.13
21	-	5.05 ± 0.18	7.17 ± 0.10

Appendix 11: Ingredients of the different Ringer's solutions for the different marine invertebrates

Echinoderms
NaCl = 6.75g
KCl = 0.23g
$CaCl_2$ = 0.31g
$MgCl_2$ = 0.50g
$MgSO_4$ = 0.15g
Distilled water = 250 ml
Mollusca
NaCl = 7.74g
KCl = 0.199g
$CaCl_2$ = 0.361g
Distilles water = 1L
Adjust to pH = 7.27

Appendix 12: Dehydration procedure followed during the preparation of histological sections

70% alcohol	40 minutes
90% alcohol	30 minutes
96% alcohol I	30 minutes
96% alcohol II	30 minutes
100% alcohol I	30 minutes
100% alcohol II	30 minutes
Xylene I	20 minutes
Xylene II	20 minutes
Xylene + wax (1:1)	45 minutes
Wax II	4 h
Wax III	90 minutes in vacuum @ 300mmHg

Appendix 13: Clearing procedure used during the preparation of histological section

Xylene I	3 minutes
Xylene II	3 minutes
100% ethanol I	Dip
100% ethanol II	3 minutes
100% ethanol III	3 minutes
96% ethanol	2 minutes
70% ethanol	2 minutes
50% ethanol	2 minutes
Distilled water	2 minutes
Hematoxylin	15 minutes
Flowing tap water	3 minutes
Scott's solution	3 minutes
1% HCl in 70% ethanol	2 dips
Flowing tap water	3 minutes
1% Eosin	30 seconds
70% ethanol	Dip
96% ethanol	3 minutes
100% ethanol	3 minutes

Appendix 14: Mean NRR times (minutes) (± SE) measured in the lysosomes of *O. tigrina* collected from different sites during different seasons (n = 5)

Sites	Winter 2000	Spring 2000	Summer 2000	Autumn 2001	Winter 2001
1	56 ±11.17	70 ± 10.09	58 ± 13.25	64 ± 7.56	53 ± 10.37
3	68 ± 12.15	89 ± 8.14	70 ±13.07	82 ± 9.63	70 ± 10.37
7	88 ± 7.50	93 ± 10.57	82 ± 8.23	99 ± 14.01	74 ± 11.01

Appendix 15: Mean NRR times (minutes) (± SE) measured in the lysosomes of *C. meridionalis* collected from different sites during different seasons (n = 5)

Sites	Winter 2000	Spring 2000	Summer 2000	Autumn 2001	Winter 2001
4	70 ± 12.94	128 ± 9.26	72 ± 9.51	130 ± 11.15	65 ± 15.79
5	72 ± 9.76	110 ± 9.50	73 ± 10.37	125 ± 11.99	70 ± 13.50
6	83 ± 9.19	140 ± 12.56	87 ± 12.28	136 ± 10.43	92 ± 9.51

Appendix 16: Mean NRR times (minutes) (± SE) measured in the lysosomes of *P. oculus* collected from different sites during different seasons (n = 5)

Sites	Winter 2000	Spring 2000	Summer 2000	Autumn 2001	Winter 2001
1	175 ± 12.64	187 ± 9.52	92 ± 7.69	133 ± 10.70	144 ± 8.23
2	163 ± 9.19	186 ± 8.63	94 ± 7.35	193 ± 7.30	167 ± 12.07
3	174 ± 10.27	154 ± 9.21	110 ± 11.55	166 ± 9.48	122 ± 10.74
4	169 ± 11.25	113 ± 10.18	99 ± 14.62	143 ± 9.84	101 ± 8.59
5	170 ± 12.48	189 ± 7.95	116 ± 6.67	158 ± 11.93	162 ± 9.47
6	150 ± 11.82	198 ± 11.95	147 ± 13.80	195 ± 12.46	136 ± 12.46
7	187 ± 11.27	200 ± 11.39	159 ± 13.16	209 ± 12.48	158 ± 13.08

Appendix 17: Mean NRR times (minutes) (± SE) measured in the lysosomes of *P. exigua* collected from different sites during different seasons (n = 5)

Sites	Winter 2000	Spring 2000	Summer 2000	Autumn 2001	Winter 2001
1	73 ± 7.11	82 ± 5.94	75 ± 9.29	81 ± 9.26	72 ± 11.60
2	84 ± 9.94	87 ± 7.01	80 ± 11.63	86 ± 8.41	81 ± 9.26
3	80 ± 11.56	88 ± 8.44	78 ± 11.52	87 ± 6.62	81 ± 9.99
4	76 ± 7.50	80 ± 10.64	71 ± 9.26	85 ± 10.49	73 ± 6.07
7	88 ± 8.20	90 9.18	77 13.97	86 8.99	82 ± 8.68

Appendix 18: Mean NRR times (minutes) (± SE) measured in the lysosomes of three experimental groups of *O. tigrina* during the 14-day exposure period and after 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	90 ± 13.81	90 ± 10.06	90 ± 9.99
3	87 ± 13.03	75 ± 8.29	70 ± 11.88
7	75 ± 7.99	65 ± 15.35	57 ± 11.80
10	70 ± 11.80	48 ± 7.16	40 ± 11.46
14	65 ± 9.40	40 ± 14.32	30 ± 11.01
21	76 ± 9.40	75 ± 14.33	50 ± 11.01

Appendix 19: Mean NRR times (minutes) (± SE) measured in the lysosomes of three experimental groups of *C. meridionalis* during the 14-day exposure period and after 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	150 ± 11.76	148 ± 8.53	148 ± 11.93
3	140 ± 11.95	130 ± 10.53	140 ± 11.01
7	132 ± 7.11	90 ± 10.07	70 ± 10.53
10	128 ± 9.15	75 ± 7.91	44 ± 9.30
14	90 ± 13.95	60 ± 10.43	30 ± 11.26
21	110 ± 11.76	72 ± 8.68	40 ± 11.46

Appendix 20: Mean NRR times (minutes) (± SE) measured in the lysosomes of three experimental groups of *P. oculus* during the 14-day exposure period and after 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	210 ± 11.76	208 ± 11.80	206 ± 14.52
3	195 ± 9.27	190 ± 12.33	180 ± 11.56
7	180 ± 13.74	160 ± 13.12	150 ± 12.64
10	175 ± 13.97	155 ± 9.73	120 ± 15.21
14	150 ± 12.20	105 ± 16.73	75 ± 9.09
21	170 ± 10.90	120 ± 12.57	90 ± 10.86

Appendix 21: Mean NRR times (minutes) (± SE) measured in the lysosomes of three experimental groups of *P. exigua* during the 14-day exposure period and after 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	90 ± 10.14	89 ± 8.56	90 ± 12.33
3	82 ± 9.60	77 ± 11.71	60 ± 11.56
7	75 ± 14.42	63 ± 9.58	30 ± 12.18
10	72 ± 11.66	45 ± 14.81	20 ± 9.93
14	30 ± 9.94	15 ± 9.65	2 ± 1.30
21	45 ± 9.89	20 ± 10.07	15 ± 9.01

Appendix 22: Mean percentage lysosomal destabilization (± SE) measured in the lysosomes of *O. tigrina* collected from the different sites during five seasons (n = 5)

Sites	Winter 2000	Spring 2000	Summer 2000	Autumn 2001	Winter 2001
1	30.1 ± 10.63	31.5 ± 8.06	30.0 ± 11.36	27.0 ± 11.45	28.2 ± 7.89
3	22.3 ± 7.21	22.0 ± 8.32	23.0 ± 8,32	23.9 ± 9.61	24.1 ± 7.37
7	21.0 ± 6.95	20.5 ± 9.80	21.00 ± 7.45	22.0 ± b9.49	23.0 ± 8.16

Appendix 23: Mean percentage lysosomal destabilization (± SE) measured in the lysosomes of *C. meridionalis* collected from the different sites during five seasons (n = 5)

Sites	Winter 2000	Spring 2000	Summer 2000	Autumn 2001	Winter 2001
4	38.4 ± 6.64	39.2 ± 9.38	37.0 ± 13.26	38.0 ± 9.90	38.1 ± 8.41
5	38.7 ± 8.99	38.3 ± 9.99	36.0 ± 12.32	37.2 ± 9.47	38.6 ± 7.24
6	30.3 ± 10.85	30.0 ± 10.28	30.1 ± 9.97	32.3 ± 10.03	34.7 ± 9.98

Appendix 24: Mean percentage lysosomal destabilization (± SE) measured in the lysosomes of *P. oculus* collected from the different sites during five seasons (n = 5)

Sites	Winter 2000	Spring 2000	Summer 2000	Autumn 2001	Winter 2001
1	25.0 ± 11.00	33.1 ± 8.10	31.0 ± 6.56	30.2 ± 11.52	34.0 ± 7.59
2	27.0 ± 6.93	24.6 ± 7.84	22.8 ± 6.87	26.0 ± 8.16	28.3 ± 9.02
3	28.0 ± 8.90	29.5 ± 9.64	27.3 ± 9.23	24.1 ± 8.82	25.7 ± 10.05
4	30.3 ± 9.22	32.3 ± 11.68	30.4 ± 8.29	31.7 ± 9.89	34.9 ± 7.84
5	34.0 ± 6.66	38.6 ± 9.99	32.6 ± 9.77	31.9 ± 7.42	35.0 ± 12.33
6	22.4 ± 7.92	20.8 ± 9.93	21.2 ± 10.72	23.6 ± 9.93	25.1 ± 7.88
7	20.0 ± 8.67	21.4 ± 9.50	19.7 ± 9.23	22.3 ± 12.31	23.8 ± 10.45

Appendix 25: Mean percentage lysosomal destabilization (± SE) measured in the lysosomes of *P. exigua* collected from the different sites during five seasons (n = 5)

Sites	Winter 2000	Spring 2000	Summer 2000	Autumn 2001	Winter 2001
1	38.0 ± 7.55	39.4 ± 8.03	36.8 ± 5.90	35.4 ± 7.49	37.3 ± 5.85
2	31.4 ± 7.49	30.0 ± 6.75	28.4 ± 6.12	30.9 ± 8.51	29.7 ± 5.67
3	36.7 ± 10.03	37.3 ± 11.32	34.4 ± 8.59	32.6 ± 6.55	38.0 ± 10.34
4	39.1 ± 9 47	36.6 ± 8.84	37.8 ± 12.47	33.6 ± 7.69	35.4 ± 6.89
7	20.2 ± 8.53	22.4 ± 6.47	21.2 ± 7.26	22.7 ± 7.86	24.2 ± 7.63

Appendix 26: Mean percentage lysosomal destabilization (± SE) measured in the lysosomes of the three experimental groups *O. tigrina* during the 14-day exposure period and following 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	20.5 ± 7.55	21.7 ± 7.36	20.4 ± 7.75
3	22.7 ± 6.49	33.4 ± 7.48	36.1 ± 8.74
7	23.4 ± 6.97	30.3 ± 9.20	31.9 ± 7.42
10	25.8 ± 10.23	34.2 ± 7.96	42.7 ± 5.56
14	26.2 ± 7.47	47.5 ± 6.69	49.0 ± 8.97
21	26.9 ± 5.29	38.4 ± 9.75	45.6 ± 11.48

Appendix 27: Mean percentage lysosomal destabilization (± SE) measured in the lysosomes of the three experimental groups *C. meridionalis* during the 14-day exposure period and following 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	31.5 ± 8.01	32.7 ± 7.51	30.6 ± 9.49
3	32.9 ± 7.98	45.0 ± 7.08	51.7 ± 8.15
7	34.6 ± 5.88	43.4 ± 5.96	49.8 ± 6.85
10	35.0 ± 7.77	49.9 ± 8.08	56.3 ± 8.15
14	37.4 ± 8.77	56.6 ± 10.77	67.7 ± 8.16
21	38.0 ± 8.32	57.2 ± 7.90	58.9 ± 11.42

Appendix 28: Mean percentage lysosomal destabilization (± SE) measured in the lysosomes of the three experimental groups *P. oculus* during the 14-day exposure period and following 1 week's decontamination 9n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	20.8 ± 8.72	20.7 ± 10.01	24.9 ± 7.13
3	22.3 ± 8.56	31.3 ± 6.89	38.1 ± 9.68
7	21.7 ± 5.78	28.9 ± 6.68	34.2 ± 5.71
10	24.3 ± 6.90	31.7 ± 7.46	41.0 ± 7.57
14	26.9 ± 6.31	58.0 ± 7.31	55.0 ± 10.77
21	27.3 ± 6.84	50.3 ± 9.25	59.6 ± 7.93

Appendix 29: Mean percentage lysosomal destabilization (± SE) measured in the lysosomes of the three experimental groups *P. exigua* during the 14-day exposure period and following 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	40.4 ± 11.25	41.5 ± 9.75	40.9 ± 5.89
3	49.1 ± 8.52	52.7 ± 7.46	53.8 ± 8.19
7	45.6 ± 9.00	49.3 ± 7.53	50.1 ± 10.36
10	46.7 ± 7.48	59.4 ± 7.74	60.5 ± 10.26
14	51.3 ± 6.88	66.3 ± 6.52	68.9 ± 7.36
21	48.2 ± 6.93	61.2 ± 8.43	65.3 ± 8.21

Appendix 30: Mean lysosomal sizes (µm) (± SE) measured in the lysosomes of the three experimental groups *O. tigrina* during the 14-day exposure period and following 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	8.90 ± 4.54	8.93 ± 4.22	8.91 ± 4.09
3	9.87 ± 4.22	10.46 ± 4.03	13.77 ± 4.97
7	10.44 ± 3.62	14.19 ± 3.82	16.15 ± 5.40
10	12.77 ± 3.96	17.63 ± 4.49	18.91 ± 5.02
14	14.66 ± 2.92	20.44 ± 4.75	22.13 ± 4.01
21	15.00 ± 4.51	22.36 ± 6.56	25.70 ± 5.49

Appendix 31: Mean lysosomal sizes (µm) (± SE) measured in the lysosomes of the three experimental groups *C. meridionalis* during the 14-day exposure period and following 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	9.74 ± 3.83	9.82 ± 3.73	9.75 ± 3.76
3	10.11 ± 3.90	13.03 ± 4.52	13.90 ± 5.35
7	12.78 ± 2.86	16.49 ± 4.69	17.30 ± 5.83
10	13.46 ± 3.85	18.90 ± 5.50	19.25 ± 6.23
14	15.17 ± 4.61	21.78 ± 6.20	22.36 ± 5.08
21	16.04 ± 4.19	25.43 ± 6.02	25.88 ± 8.18

Appendix 32: Mean lysosomal sizes (µm) (± SE) measured in the lysosomes of the three experimental groups *P. oculus* during the 14-day exposure period and following 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	6.10 ± 3.66	6.60 ± 3.97	6.58 ± 3.43
3	7.17 ± 3.38	9.76 ± 4.50	9.81 ± 3.60
7	8.49 ± 4.49	11.77 ± 5.40	12.15 ± 5.42
10	10.47 ± 5.53	14.69 ± 3.86	16.17 ± 5.16
14	13.99 ± 4.91	17.70 ± 3.28	19.70 ± 3.46
21	14.54 ± 4.16	19.97 ± 5.85	24.24 ± 3.58

Appendix 33: Mean lysosomal sizes (µm) (± SE) measured in the lysosomes of the three experimental groups *P. exigua* during the 14-day exposure period and following 1 week's decontamination (n = 5)

Exposure time (days)	Control	200 µg/L	400 µg/L
0	5.79 ± 2.94	5.84 ± 3.22	5.88 ± 2.42
3	6.94 ± 2.76	7.22 ± 2.73	12.44 ± 6.36
7	7.67 ± 3.50	10.14 ± 6.22	15.66 ± 4.69
10	9.04 ± 5.61	13.17 ± 5.62	17.84 ± 4.42
14	13.44 ± 6.16	14.77 ± 4.83	19.46 ± 6.84
21	14.69 ± 7.07	15.66 ± 4.46	21.34 ± 4.99

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